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## ORIGINAL ARTICLES

### THE TECHNIC OF BASAL METABOLIC RATE DETERMINATION IN PSYCHONEUROTIC PATIENTS*


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IN MAKING basal metabolic rate determinations of psychoneurotic persons, several technical points must be observed, in order that more efficient results may be obtained. The general appearance of the apparatus, for instance, should present as simple an arrangement as possible, so that it may arouse no suspicion in the mind of the patient that he is to be subjected to a complicated procedure. It is best that the pipes and most of the connections be concealed from view, and that the bed upon which the patient is to rest appear but little different from the hospital cot to which he is accustomed.

To attain this end, the following arrangement has been adopted at this hospital in connection with the Tissot-Haldane combination. An ordinary hospital folding cot is used as the bed. It is placed in the corner of a quiet room with the foot end close to a window. The intake air pipe, *A* (Fig. 1), is fastened to the bed under the spring in a way which completely conceals it from view. At the foot end, the pipe is directed vertically upward for about 5 to 7 inches by means of an elbow, and by use of another elbow it is extended out horizontally for about 10 to 12 inches. This horizontal extension, *A* (Fig. 2), passes through a hole in a board about seven inches wide, placed in the window in a manner similar to that of a window screen. The lower part of the window rests directly over the top edge of the board sufficiently tight to prevent the wind from blowing in at the junction. If necessary, a strip of felt may be nailed at the junction in the winter.


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The pipe passes through the board as near the window sill as possible, and is completely concealed from view when the bed is made up.

The intake air pipe is directed upward at the head end of the bed also by the use of an elbow, just sufficiently to enable the fastening over it of a flexible ribbed rubber hose. This projection, *B* (Fig. 1), is tapered at the end, so that the hose may be attached to it with facility. At the other side of the head end of the cot is fastened an arrangement which connects the spirometer to the "Expiration Hose." Its construction is shown in Fig. 3. *A* is a T-shaped connection, *B* are the horizontal short arms of the *T* made of short pipe-pieces tightly screwed into the *T* and tapered at the ends, to facilitate placing the hose connections over them. *C* is the vertical long arm made of a pipe 24 inches long, screwed into the *T*, and which is used merely as a holder. The top opening of *C* is tightly plugged before it is connected with the *T*, to prevent the escape of the expired air. The entire combination is fastened by means of its long arm to the head-end of the cot, opposite the

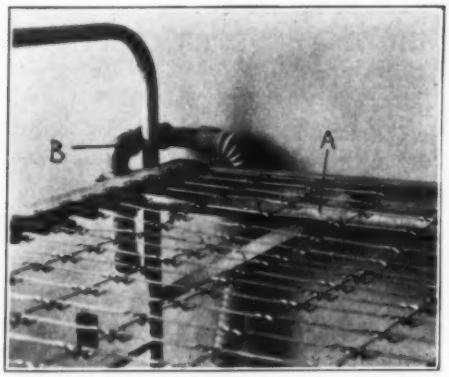


Fig. 1.

Fig. 2.

intake connection, just high enough to allow slipping over it the flexible ribbed "Expiration Hose," *D*, yet low enough to conceal it when the bed is made up.

The device used for the control of the inspiration and expiration is a point of next importance. The gas mask, as described by Boothby and Sandiford,[1] has been in use for that purpose at this hospital for several months, and as a result, the following objections were brought out. Nearly all the patients at the hospital had some experience with gas masks, and many of those tested manifested a definite reaction, due to the associations brought about by the mask, affecting the end-result markedly. Patients possessed of psychopathic tendencies and lacking the natural intelligence necessary for the proper cooperation in the carrying out the B.M.R. determination procedure, could not keep still when the mask was fastened over their faces. Indeed, they were invariably so restless that the mask shifted, causing considerable leakage, and resulting in such variations in the final rates, that it was difficult to come to a conclusion as to which was the

[1]Laboratory Manual of the Technic of B. M. R. Determinations, W. B. Saunders Co., Philadelphia.

normal rate of the patient. In one case, where the patient voluntarily came for rate determinations six times, no two rates obtained were sufficiently close to be called duplicates.

Many of the confirmed hysterics and of the anxiety patients behaved much the same as did the psychopaths for the first two or three tests, but finally became accustomed to the procedure, though they never expressed a liking for it.

Finally, in cases with certain facial deformities, caused by wounds while in action, proper adjustment of the mask was impossible, in spite of all its flexibility. The use of the mask had to be abolished, and the mouth piece and nose clip were tried out. About fifty patients, previously tested by the use of the gas mask, were retested by the mouth piece and nose clip method, and all but one expressed the unsolicited preference for the latter. The one differing in the opinion was a woman, (army nurse), who had a

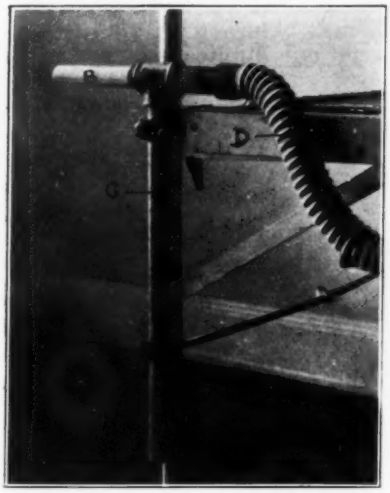


Fig. 3.

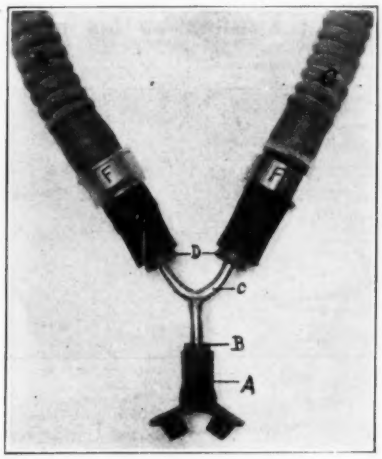


Fig. 4.

distinct thyroid envolvement with a consistent rate of plus 57 per cent. This resulted in the exclusive adoption of the mouth piece and nose clip, although masks are had in store to accommodate a particular necessity which may arise.

The arrangement now in use is shown in Fig. 4. *A* is the mouth piece, *B* is a No. 1 rubber stopper, *C* is a nickel-plated metallic Y-shaped tube, *D* are No. 5 rubber stoppers, *E* are short pieces of gardener's rubber hose. *F* are the controlling air valves, and *G* the flexible ribbed rubber hose.

In carrying out a determination on new cases where it is suspected an objection or a lack of proper cooperation may be encountered, the following procedure is resorted to, which may be described as consisting of three steps.

1. With the mouth piece, Y-shaped metallic tube and the rubber stoppers detached from the rest of the arrangements and with the ribbed rubber hose and valves connected to the intake and expiration pipes, but properly concealed from view, the patient is placed on the bed, and his temperature,

respiration rate, and blood pressure are taken. These are procedures to which he has been subjected before, and almost invariably induce the patient to display a certain amount of confidence and cooperation. After the required resting period has elapsed, the mouth piece is introduced, and is properly propped up by a small pillow as is shown in Fig. 5, and the patient is allowed to rest in this position for a minute or two.

2. The nose clip is fastened over the nose, as is shown in Fig. 5, and the patient is allowed time to adjust his respiration rhythm.

3. The flexible ribbed rubber connections are attached to the corresponding ends of the Y-shaped tube, as is shown in Fig. 6, and the determination is completed in the usual manner.

Where proper cooperation on the part of the patient is expected, or when a repeat determination is carried on, the mouth piece is connected to the ribbed hose while the patient is resting, and the attached mouth piece introduced into the mouth, the nose clip fastened, and the determination proceeded with at once.

It is the custom at this institution to carry out duplicate determinations

Fig. 5.

Fig. 6.

in succession, to rule out possibilities of errors in the procedure. To this end, the nose clip alone is usually removed at the completion of the first determination, and the patient is allowed to breathe through the nose, but with the mouth piece in the mouth, while the readings and the temperature of the spirometer are taken and the samplers are filled. As soon as this is done, and the spirometer is emptied, the nose clip is replaced, and the duplicate determination proceeded with at once.

This was found to save considerable time and annoyance to both the laboratorian and to the patient. In individual cases, the mouth piece as well may be removed, if such a procedure is indicated, but the writer has found only two out of fifty cases in which such a procedure seemed wise, though not necessary.

At the end of a duplicate determination the mouth piece is detached, washed with soap and hot water, sterilized in alcohol or carbolic acid, again thoroughly rinsed with hot water, and used as before. This can be done while the patient is resting; or two mouth pieces may be had, so that one may be washed and sterilized, while the other one is used.

As stated above, the Tissot-Haldane combination has been in use at this

institution, although other combinations, considerably simpler in appearance and in manipulation are now obtainable. It should be stated, however, that the Tissot-Haldane principles yield results which may be designated as dependable even for scientific purposes. The additional data, such as the variations in the Respiratory Quotient variations in the ventilation, and in the nature of the respiration, which the Tissot-Haldane combination affords the investigator, have been found highly useful in differentiating types of neurotic patients, even when their rates stayed within the normal limits. If properly observed and properly interpreted, this procedure of Basal Metabolic Rate Determination more than compensates the diagnostician for the extra work it demands.

Grateful acknowledgment is made to Surgeon Lawrence Kolb, Medical Officer in Charge of this Hospital, for his permission to prepare and publish this paper; to P. A. Surgeon (R) C. Covey, Clinical Director, for his general assistance, and to Mr. D. E. Roberts of this Hospital for preparing the photographs reproduced herein.

# COMPARATIVE CONCENTRATION OF UREA IN THE BLOOD AND SALIVA IN A SERIES OF PATHOLOGIC CASES*

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IT HAS long been established that urea diffuses very readily through cell membranes. Red blood cells placed in a urea solution almost instantly acquire the same concentration of urea as that of the solution itself, without any noticeable injurious effect upon the cells themselves.[1] The work of Marshall and Davis[2] has shown that urea is found in all tissues and body fluids in approximately uniform concentration. This uniform distribution also occurs when the urea content of the body is increased, either by intravenous injections of urea, or by defective elimination of this substance, or by both. Cullen and Ellis,[3] and Myers and Fine[4] have pointed out the remarkably close relationship between the concentration of urea in the blood and spinal fluid. As a result of these observations it appeared of interest to study the relationship between the concentration of urea in the blood and saliva in various pathologic conditions.

Urea has been regarded as a constituent of normal saliva for many years. Rabuteau[5] in 1873 observed that after taking 5 grams of urea and 200 c.c. of water, the salivary secretion was markedly increased, and that the saliva had a peculiar taste. It immediately occurred to him that this peculiar taste might be due to urea secreted in the saliva. This fact he verified by chemical analysis, 35 c.c. of saliva being found to contain 0.0235 grams of urea. An analysis of normal saliva made at this time also showed the presence of this compound. Rabuteau, however, was not the first to detect urea in saliva. Samuel Wright[6] in 1841 reported a "Case of Ascites in Which During a Spontaneous Ptyalism That Occurred after Tapping, Urea Was Detected in the Saliva." The secretion was chocolate in color, slightly ammoniacal in odor, and contained 130 mg. of urea per 100 c.c. On two previous occasions Wright demonstrated urea in the saliva of dogs poisoned by corrosive sublimate. Von Zerschwitz[7] in 1883 studied the urea content of saliva in normal persons and in cases of nephritis. He concluded that the determination of urea in saliva in normal individuals was practically impossible, even with their most delicate methods since only occasionally traces of this compound could be detected with the amyl-alcohol method; but that in cases of nephritis, its detection in saliva was comparatively simple and accurate, and that the urea curve varied in direct proportion to the intensity of the kidney lesion. How-

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Possibilities of the utilization of the determination of the concentration of the urea in the saliva was simultaneously discussed by Drs. Rowntree and Myers at the Seventy-Third Meeting of The American Medical Association, June, 1922 (Jour. Am. Med. Assn., 1922, lxxix, 1389-1390). Since that time and after our paper was submitted for publication, Hench and Aldrich in Dr. Rowntree's Department at the Mayo Clinic have given a brief discussion of this subject (Jour. Am. Med. Assn., 1922, lxxix, 1409).

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ever, no attempt was made to express the urea content in terms of grams or milligrams, the terms, traces, small and large amounts of urea crystals, being employed. Since the methods used by these early investigators were of questionable accuracy, their results must be taken with reserve.

Marshall and Davis, in their analysis of the urea distribution in the various tissues and body fluids, found that the urea concentration of the parotid gland was exactly the same as that of the blood, although they reported no urea determinations on the mixed saliva.

Some of our determinations (made by Dr. Myers) were begun during the early part of 1922. The observations include the urea content of both blood and mixed saliva and ammonia in saliva. The blood and saliva specimens were obtained the same day, except as indicated in the table. The estimation of urea was performed by the method described by Myers.[8] In case of the saliva, 2 c.c. of the filtered secretion were used for the analysis. As we shall see later, the urea in the saliva as determined by this method, represents the combined value of both urea and ammonia. The ammonia was determined by the same method, omitting the addition of urease and incubation.

## METHOD OF COLLECTING SALIVA

The teeth are carefully brushed, and the mouth thoroughly rinsed out with water. As a salivary stimulant, the patient is given a small piece of paraffin to chew, and all the saliva secreted is collected in a small clean cup to which 2-3 drops of molecular phosphate have been added. The determinations are made immediately after the specimens have been collected.

## DISCUSSION

At the time this work was begun, the saliva was also analyzed for ammonia, and it was found to contain variable amounts of this compound. The amount of ammonia present did not seem to bear any definite relation to the combined amount of urea and ammonia, for in some cases it almost equaled, but in others only represented a small fraction of the total amount of urea and ammonia. Any relationship between the ammonia concentration of the blood and saliva would appear extremely improbable, since the concentration in the blood is so much lower and so much more constant.[9]

TABLE I

AMOUNT OF AMMONIA IN SALIVA AFTER TEETH AND MOUTH HAD BEEN THOROUGHLY CLEANED

| DETERMINATION | UREA PLUS AMMONIA N | AMMONIA N |
|---|---|---|
| | MG. PER 100 C.C. | |
| 1 | 11.1 | 6.5 |
| 2 | 5.4 | 4.4 |
| 3 | 9.8 | 4.6 |
| 4 | 8.2 | 4.3 |
| 5 | 73.5 | 11.5 |
| 6 | 67.7 | 7.6 |
| 7 | 111.9 | 5.3 |

Possible sources of ammonia are: (1) From the saliva, secreted as a normal constituent. (2) From putrefaction of food particles present between the teeth. (3) From conversion of urea to ammonia due to bacterial activity.

That ammonia is not a normal constituent of parotid saliva is evidenced by the fact that specimens of parotid saliva obtained by means of a cannula inserted into Stenson's duct did not show the presence of this compound. Specimens of saliva collected after the teeth had been carefully brushed and the mouth thoroughly rinsed were analyzed immediately for ammonia, and were found to contain small amounts of this substance in every instance (Table I). The probability of the ammonia being due to putrefaction of food particles is practically removed by this test. To determine whether or not the ammonia was derived from urea, specimens of saliva were analyzed immediately for urea and ammonia and again after standing for several hours. As shown in Table II, it is evident that the combined urea and ammonia values decreased and the ammonia values increased. The increase in ammonia must have taken place at the expense of the urea, for the latter compound was considerably reduced in amount. When 2-3 drops of molecular phosphate were added, there was no change in the combined value of urea and ammonia, but the ammonia had increased considerably and the urea decreased proportionately, supporting the assumption that the urea is the source of ammonia in mixed saliva (Table II).

TABLE II

SALIVA ANALYZED IMMEDIATELY FOR UREA AND AMMONIA AND AFTER STANDING SEVERAL HOURS

| SPECIMEN | ANALYZED IMMEDIATELY | | ANALYZED AFTER 8 HRS. | | ANALYZED AFTER 24 HRS. | |
|---|---|---|---|---|---|---|
| | UREA PLUS $NH_3$–N | $NH_3$–N | UREA PLUS $NH_3$–N | $NH_3$–N | UREA PLUS $NH_3$–N | $NH_3$–N |
| | MG. PER 100 C.C. | | | | | |
| 1 | 73.5 | 11.5 | 65.8 | 22.0 | 40.0 | 17.0 |
| 2 | 83.1 | 11.8 | | | 74.2 | 24.7 |
| *3 | 67.0 | 7.4 | | | 65.7 | 17.6 |
| *4 | 26.3 | 14.9 | | | 26.7 | 25.6 |
| †5 | 28.8 | 5.0 | | | 28.2 | 4.2 |

*Molecular phosphate added to saliva.
†Saliva run through Berkefeld filter.

A specimen of saliva to which molecular phosphate had been added was run through a Berkefeld filter and collected in a sterile flask. The filtered saliva was immediately analyzed for urea and ammonia, and again after twenty-four hours. Both determinations gave practically the same results (Table II), thus suggesting the possibility that the greater part of the ammonia content of saliva is derived from urea, the result of bacterial activity and not as the result of an enzyme.

Table III presents forty-five simultaneous determinations of urea in the blood and saliva in various pathologic conditions. The observations are listed according to the amount of urea retention in the blood. It will be noted that in the majority of cases, the urea content of the saliva runs somewhat lower than that of the blood, but that as the urea concentration of the blood is increased, the urea value of the saliva likewise rises. In the forty-five determinations, the average salivary urea content amounts to 89.4 per cent of that of the blood. Myers and Fine[4] found that the concentration of urea in spinal fluid averaged 88 per cent of that of the blood.

TABLE III

COMPARATIVE CONCENTRATION OF UREA IN THE BLOOD AND SALIVA

| CASE | AGE | SEX | BLOOD | SALIVA | | REMARKS |
|---|---|---|---|---|---|---|
| | | | UREA N | UREA PLUS AMMONIA N | AMMONIA N | |
| | | | MG. PER 100 C.C. | | | |
| 20916 | 47 | M | 10.7 | 11.2 | | |
| 21707 | 52 | M | 10.7 | 8.8 | 6.5 | Chronic Cholecystitis, Cholelithiasis. |
| 21676 | 34 | M | 12.1 | 10.8 | 10.3 | Ventral Hernia. Intraabdominal Adhesions. |
| 21001 | 52 | M | 15.2 | 16.1 | .... | Cerebro-Spinal Lues. Chronic Plumbism. Mitral Regurgitation. Hypertension. |
| 21766 | 53 | M | 15.0 | 13.0 | 9.7 | Chronic Appendicitis with Adhesions. |
| 21702 | 44 | F | 15.3 | 19.5 | .... | Diabetes Mellitus. |
| 21806 | 48 | M | 15.4 | 12.5 | 8.6 | Carcinoma of the Esophagus. |
| 21211 | 34 | M | 15.6 | 11.5 | .... | Hyperthyroidism. |
| 21935 | 20 | M | 15.9 | 10.0 | 4.7 | Psycho-neurosis. |
| 21975 | 48 | M | 16.0 | 17.5 | .... | Chronic Cholecystitis. |
| 21836 | 34 | F | 16.5 | 10.1 | 5.0 | Psychoneurosis. Obesity. |
| 21975 | 48 | M | 16.5 | 17.2 | 12.5 | Chronic Cholecystitis. |
| 21975 | 48 | M | 16.5 | 17.7 | .... | Chronic Cholecystitis. |
| 21639 | 46 | F | 17.5 | 14.8 | 7.3 | Nephrosis. Chronic Myocarditis. |
| 21771 | 53 | M | 18.1 | 10.5 | 8.5 | Prostatic Obstruction. Vesical Calculus. |
| 21229 | 60 | M | 18.2 | 14.5 | .... | Lues. |
| 20438 | 45 | M | 18.7 | 22.5 | 17.7 | Chronic Nephritis. Diabetes Mellitus. |
| 22170 | 70 | M | 20.2 | 17.8 | 13.1 | Diabetes Mellitus. Edematous Prostate. |
| 20760 | 54 | M | 20.2 | 18.7 | 16.9 | Chronic Nephritis. Myocardial Insufficiency. |
| 22170 | 70 | M | 21.7 | 20.6 | .... | Diabetes Mellitus. Edematous Prostate. |
| 21008 | 49 | M | 21.8 | 18.3 | .... | |
| 20071 | 60 | M | 22.3 | 20.6 | 11.6 | Prostatic Hypertrophy. |
| 15526 | 31 | M | 22.6 | 17.0 | 10.0 | Chronic Parenchymatous Nephritis. |
| 21864 | 45 | M | 23.0 | 19.7 | 17.7 | Diabetes Mellitus, Cerebrospinal Lues. |
| 20438 | 45 | M | 23.2 | 20.2 | 10.0 | Diabetes Mellitus. Chronic Nephritis. |
| 22088 | 53 | M | 25.6 | 31.1 | .... | Essential Hypertension. |
| 21498 | 62 | M | 30.7 | 25.1 | .... | Prostatic Obstruction. |
| 20071 | 60 | M | 30.9 | 22.7 | 15.9 | Prostatic Hypertrophy. |
| 20071 | 60 | M | 34.8 | 26.3 | 14.9 | Prostatic Hypertrophy. |
| 21240 | 47 | M | 35.8 | 27.5 | .... | Chronic Interstitial Nephritis. Nephrolithiasis. |
| 21498 | 62 | M | 37.4 | 37.1 | .... | Prostatic Obstruction. |
| 21498 | 62 | M | 39.7 | 42.1 | .... | Prostatic Obstruction. |
| 20071 | 60 | M | 42.8 | 28.5 | .... | Prostatic Hypertrophy. |
| 21498 | 62 | M | 46.8 | 43.5 | .... | Prostatic Obstruction. |
| 16515 | 55 | M | 60.0 | 48.0 | 20.0 | Arteriosclerotic Kidneys. Hypertension. |
| 19412 | 26 | M | 61.4 | 45.5 | .... | Chronic Interstitial Nephritis. |

TABLE III—CONT'D

COMPARATIVE CONCENTRATION OF UREA IN THE BLOOD AND SALIVA

| CASE | AGE | SEX | BLOOD | SALIVA | | REMARKS |
|---|---|---|---|---|---|---|
| | | | UREA N | UREA PLUS AMMONIA N | AMMONIA N | |
| | | | MG. PER 100 C.C. | | | |
| *17135 | 55 | M | 61.5 | 40.0 | 30.0 | Arteriosclerotic Kidneys. Hypertension. Cardiac Hypertrophy. Dilated Aorta. |
| 19412 | 26 | M | 62.5 | 55.5 | 23.1 | Chronic Interstitial Nephritis. |
| 19271 | 34 | M | 64.1 | 43.6 | .... | Chronic Nephritis. Chronic Secondary Anemia. |
| *17177 | 53 | M | 65.0 | 79.0 | 10.0 | Polycystic Kidneys. Myocardial Insufficiency. |
| *16753 | 50 | M | 74.9 | 83.0 | 60.0 | Chronic Interstitial Nephritis. Cardiac Hypertrophy and Dilatation. |
| 19412 | 26 | M | 75.0 | 50.0 | .... | Chronic Interstitial Nephritis. |
| 19412 | 26 | M | 86.2 | 73.5 | 11.5 | Chronic Interstitial Nephritis. |
| 19412 | 26 | M | 98.0 | 98.7 | .... | Chronic Interstitial Nephritis. |
| 22065 | 65 | F | 100.0 | 111.9 | 5.3 | Chronic Interstitial Nephritis. |

*Specimens not collected on the same day.

Since the saliva acquires practically the same urea concentration as the blood, salivary urea determinations will, therefore, give us definite information in pathologic cases with urea retention. In cases where it is impossible or impracticable to obtain specimens of blood, a determination of the urea concentration of the saliva will serve as a fair index of the functional activity of the kidneys.

## SUMMARY

Comparative simultaneous analyses on forty-five specimens of blood and saliva, representing various pathologic conditions, are reported. The urea content of saliva averaged 89.4 per cent of that of the blood. Salivary urea determinations may be employed in determining the functional activity of the kidneys where for any reason it is impracticable to obtain blood specimens.

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# A REPORT OF SEVEN CASES OF NICOTINE POISONING*

By Wm. D. McNally,† A.B., M.D., Chicago, Ill.

POISONING by the alkaloid nicotine is increasing, due to its more frequent use as an insecticide in recent years. The commercial preparations containing from 8 to 43 per cent of the alkaloid nicotine are used in very dilute solutions as insecticides. The free alkaloid is a colorless oily liquid which rapidly becomes brown closely resembling whisky in appearance which accounts for its accidental consumption.

In a previous paper (Jour. Lab. and Clin. Med., 1920, iv, No. 4) I reported five cases of poisoning by this alkaloid. Since that time material has been submitted from seven other deaths; viz., organs in four instances and bottles of insecticides in the three others. There were verdicts, "suicide" in four, "accident" in two, and in one "undetermined." Five of these people were found dead, in the remaining two it is known that death occurred in less than five minutes. A brief history of each follows:

No. 1, J. A. Nov. 6, 1919, took ½ oz. of an insecticide, which upon analysis contained 40.86 per cent nicotine. After taking the poison he walked up a flight of stairs from the basement of his home to the kitchen, where he vomited; he then walked about twenty feet to his bedroom and dropped dead. The time estimated by his family was less than five minutes after he started to walk from the basement until death occurred. There was no autopsy. The Coroner's verdict read "took nicotine with suicidal intent while temporarily insane."

No. 2, E. H. P. Sept. 22, 1919, (Footnote, The newspaper publicity given this case may have been responsible for other poisonings by nicotine, as was previously noted in deaths by mercuric chloride, McNally, W. D., Med. Rec., Feb. 1, 1919). A camouflaged breakfast and robbery had been staged to give the appearance of murder. The deceased was found gagged and bound in a large armchair in the kitchen of his home. Upon the handkerchief gag and upon his shirt were brown stains; on the floor there was a broken tumbler. The ropes binding the arms were not securely tied on the left arm, and the right arm had 9½ inches of slack, which was sufficient to permit a person to slip down in the chair and drink from a glass tumbler. The postmortem examination of the body demonstrated a hyperemia of all the organs, otherwise nothing grossly was observed, except a peculiar odor in the stomach. A chemical examination of the organs revealed the presence of nicotine. (See Table for amounts). Nicotine was found on the gag and bosom of shirt. In the coffee and milk on the table tests failed to show the presence of nicotine. The brain contained none, the kidneys and liver gave only traces. The authorities were undetermined as to whether this was a suicide or murder.

No. 3, D. D., (male), March 11, 1920, had been drinking heavily and took 1 oz. of an insecticide containing nicotine. No autopsy was held. The verdict of the Coroner's jury was given as "suicide while despondent and temporarily insane." An empty 1 oz. bottle which had contained nicotine was submitted.

No. 4, G. S., May 7, 1920, is said to have taken nicotine by mistake for cascara and

*Received for publication, April 11, 1922.
†Chemist to the Coroner of Cook County, Chicago, Ill.

TABLE NO I.

NICOTINE IN MILLIGRAMS

| | LAB. NO. | STOMACH | | STOMACH CONTENT | | STOMACH AND CONTENT | | BOWEL | | LIVER | | KIDNEY | | URINE | | BRAIN | | NICOTINE BOTTLE LIQUID % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | In total weight material rec'd. | In 100 grams | In total weight material rec'd. | In 100 grams | In total weight material rec'd. | In 100 grams | In total weight material rec'd. | In 100 grams | In total weight material rec'd. | In 100 grams | In total weight material rec'd. | In 100 grams | In total weight material rec'd. | In 100 grams | | | |
| E. H. P. Undetermined | 5175 | In 138 G. 990.50 mg. | 717.60 mg. | | | | | In 807 G. 782.70 mg. | 97.00 mg. | Trace | | Trace | | | | Negative | | |
| G. S. Accident | 6108 | In 224 G. 441.79 mg. | 200.70 mg. | In 310 c.c. 3184.2 mg. | 1271.7 mg. | | | | | | | | | | | | | |
| M. M. Suicide | 6387 | | | | | In 282 G. 1610.2 mg. | 571.0 mg. | In 276 G. 9.6 mg. | 3.5 mg. | In 173 G. 9.1 mg. | 5.3 mg. | In 135 G. 14.4 mg. | 10.7 mg. | In 280 c.c. 1.1 mg. | 0.4 mg. | | | 20.06 % |
| M. V. | 7019 | In 161 G. 18.5 mg. | 11.4 mg. | | | | | In 478 G. 65.1 mg. | 13.6 mg. | In 1255 G. 37.0 mg. | 2.9 mg. | In 372 G. 16.1 mg. | 4.6 mg. | | | | | |

was found dead in bed with no glass or bottle near. The postmortem demonstrated a hyperemia of all the organs as already noted. A chemical examination of the organs revealed that death was due to nicotine poisoning. At the inquest a bottle of nicotine was produced which was said to have been found in the clothes hamper in the basement. The results of the chemical examination can be seen in the Table.

No. 5, M. M., July 19, 1920, a housemaid took an unknown quantity of a bottle labeled "Nikoteen" containing 28.60 per cent nicotine. She had been drinking heavily and when informed that her services as housemaid were no longer required, took the poison and was found dead the next morning. A postmortem examination was made and the organs submitted for chemical examination.

A verdict of "suicide while temporarily insane" was rendered. Investigation revealed the fact that the deceased had been confined in an asylum for four months the year prior to her death.

No. 6, F. R. D., Sept. 12, 1919, a boy seven years of age, was given one-half a dram of an insecticide in mistake for a cough mixture. The bottles were practically of the same design, both containing dark brown liquid. The child walked 18 feet after taking the liquid, death occurring in less than five minutes. There were no organs submitted. A chemical examination of the insecticide gave 31.55 per cent nicotine.

No. 7, M. V., Los Angeles, was found dead the morning of April 5, 1921 and the body was shipped to Chicago. The mysterious circumstances surrounding this death caused exhumation of the girl's body, April 10, 1921. The death certificate gave the cause of death as "diphtheria," evidence of which could not be seen by the pathologist here at the autopsy. The postmortem showed that an abortion had been recently performed. A chemical examination of the organs revealed that death was due to nicotine poisoning.

In all of the postmortem examinations mentioned, no disease from natural processes was found which in any way contributed to death.

During a toxicologic investigation of several thousand deaths, it has been noted that in certain embalmed bodies that the alkaline distillate from the steam distillation of the tissues gives several of the reactions for nicotine. The results of the research upon the alkaline distillate will be the subject of a subsequent paper in preparation.

# THE LOCAL WASSERMANN IN THE EARLY DIAGNOSIS OF PRIMARY SYPHILIS*

By D. Stern, M.D., and Harold Rypins, M.D., Minneapolis, Minn.

## INTRODUCTION

THE POSITIVE diagnosis of syphilis in the early primary stage of the disease, before the occurrence of systemic invasion, is of the utmost importance in prognosis and treatment. But it is in the primary stage that an absolute diagnosis is sometimes most difficult. In the early stages, the blood Wassermann reaction is usually negative. The presence of *Spirochete pallida* in the lesion is the only positive criterion of a primary chancre. Occasionally, no dark-field microscope is available. A certain percentage of cases which later develop positive blood Wassermanns, fail to show spirochetes in the lesion on repeated careful examinations. Lastly, lesions which have been treated locally with a spirocheticide, usually show no spirochetes. Consequently, because of both the great importance of an early diagnosis, and the absence of any single infallible method, an additional means of diagnosing a primary chancre is of inestimable value. In certain cases, the "local" Wassermann reaction, performed on the surface serum of a suspected primary chancre, appears the only means of making a positive diagnosis of syphilis, at the time when a clear-cut diagnosis is of greatest value.

Within the past year, Klauder and Kolmer[1] have reported the results of studies on the Wassermann reaction carried out on various body secretions, as well as transudates and exudates, particularly with reference to the site of origin of the specific complement-fixing antibodies. To demonstrate the origin of specific complement-fixing antibodies locally as well as hematogenously, these workers studied the Wassermann reaction of the surface fluid from a number of primary chancres. These tests "yielded almost uniformly a four-plus reaction. In some instances, positive results were obtained before the test appeared positive in the blood, which apparently excludes the possibility of the positive reaction in the chancre fluid being due to admixed blood." Since the completion of our present study, Klauder and Kolmer[2] have published the results of the local Wassermann on 14 cases, and have elaborated the suggestion contained in their first paper of the practical value of the local Wassermann as an aid in the early diagnosis of syphilis. Their results will be discussed in comparison with our own findings below.

## MATERIAL AND METHODS

*Material.*—Most of the cases in this series were referred for study through the courtesy of the members of the Department of Dermatology and Syphilis

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of the Medical School, the remainder coming to the State Board Laboratory from various physicians for dark-field examinations. Although only cases in which the diagnosis of primary chancre appeared probable were studied, every case in which the local Wassermann test was performed, is included, so that the remarkably constant results are not the distorted effect of omitting any negative reactions. The presence of *Spirochete pallida* is taken as the only unquestionable criterion of a true primary chancre. The usual clinical data, including the time from exposure to the appearance of the lesion, the time since the appearance of the lesion, the presence of satellite or general adenopathy, the occurrence of secondary lesions, and the blood Wassermann at the time of examination are included to complete the clinical picture.

*Technic.*—The surface of the chancre is sponged off with normal saline, after which it is dried and gently squeezed. Ordinarily, sufficient serum exudes without difficulty. If there is not enough serum present, the surface of the lesion is scratched lightly with the end of a glass capillary tube, since the admixture of a little blood in no wise interferes with the reaction. The surface serum is collected by capillary suction in a fine glass tube, graduated to 0.1 c.c. From one to two tenths of a cubic centimeter of serum are usually obtainable. A 0.1 c.c. drop of the serum was used in all cases. Dilutions of 1 to 8, 1 to 16 and 1 to 24, giving serum in amounts of 0.0125, 0.00625 and 0.004 c.c., respectively, were made up to the extent of the serum obtained. The routine Wassermann technic used by the laboratory of the Venereal Division of the Minnesota State Board of Health, which during a corresponding length of time was used in about 49,000 blood Wassermann tests, was employed in all cases. This is the standard antisheep cell hemolytic system, using a single, noncholesterinized, alcoholic extract of human heart for antigen. Controls for anticomplementary action of the chancre serum were negative in all cases.

## RESULTS

*Blood Wassermanns.*—Thirty-four cases of demonstrated primary chancre and two with proved nonluetic lesions are included in this series. The clinical and laboratory findings are tabulated in Table I, and summarized in Table II. For convenience, the cases are divided into four groups: Group I: Ten cases with positive dark-field, positive blood Wassermann and positive local Wassermann. Group II: One case with positive dark-field, positive local Wassermann and weakly positive blood Wassermann. Group III: Twenty-three cases with positive dark-field, positive local Wassermanns but negative blood Wassermanns. Group IV: Two cases with all the laboratory findings negative, which subsequent observation demonstrated as nonluetic in character.

Thus in thirty-four cases of primary chancre in which the local Wassermann reaction was positive, only ten exhibited a positive blood reaction, one case being weakly positive and the remaining twenty-three cases showing a negative reaction in the blood serum. These findings agree with the results

of Klauder and Kolmer, who found that five cases out of twelve having both positive dark-fields and positive local Wassermanns, exhibited negative Wassermann reactions with the blood serum. As early as the third day after the appearance of the primary chancre, we found a positive reaction with the chancre serum. Kolmer and Klauder report a case of a seven day chancre with the local Wassermann reaction positive, in which the blood reaction was negative with three antigens. These cases strongly support the conception of a local, as well as hematogenous, origin of the specific complement-fixing antibody.

*Presence of Spirochetes.*—Strikingly constant is the occurrence of a positive local Wassermann reaction without exception in every case of demonstrated primary chancre. The local Wassermann parallels the dark-field findings, and is independent of the blood Wassermann reaction, the presence or absence of adenopathy or secondary manifestations, or the duration of the chancre itself. These findings suggest that the local complement-fixing body may occur only in the presence of *Spirochete pallida* in the lesions, but Klauder and Kolmer report five cases yielding positive local Wassermann reactions in which spirochetes were not demonstrable in the chancre. The parallelism between the presence of spirochetes and a positive local reaction can be best interpreted by concluding that the local Wassermann reaction is positive in a very high percentage, if not all cases, of true primary chancre.

*Effect of Local Treatment.*—The effect of local treatment is illustrated by case 17, which had been treated with iodoform, but still gave a positive reaction with the chancre fluid. In this case the spirochetes were still present, but it is usually difficult for spirochetes to be found from the lesion which has been treated locally. Klauder and Kolmer report a case in which the spirochetes were absent but in which the chancre fluid gave a positive reaction, although the lesion had been treated with a spirocheticidal drug. It is in this type of case that a positive local Wassermann reaction would be the only certain diagnosticating finding.

*Dilution of Serum.*—In amounts of 0.1 and 0.0125 c.c. the local Wassermanns were uniformly positive; with only 0.00625 c.c. there were twenty positives, six weak positives and ten negatives; with 0.004 c.c. there were only two positives, five weak positives and twenty negative reactions. Taking into consideration the varying dilution of the chancre serum with the saline with which the lesion is washed, the amount of serum fixing complement is comparable to the amount giving a positive reaction with blood serum, that is, approximately 0.001 c.c. Since positive reactions were obtained uniformly with 1 to 8 dilutions of the original 0.1 c.c., it seems reasonable to suppose that obtaining the serum by adding four of five drops of saline to the surface fluid and then pipetting off the mixture would give a strong enough concentration of antibodies to yield positive reactions.

## SUMMARY

The Wassermann reaction carried out on the surface serum of a suspected primary lesion is a practicable and valuable aid in the early diagnosis

TABLE I

| CASE | | PRIMARY CHANCRE | | | | | BLOOD WASS. | DARK FIELD | WASSERMANN ON CHANCRE SERA | | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DAYS* DURATION | DAYS** EXPOSURE | LOCATION | ADENOPATHY | SECONDARY LESIONS | | | 0.1 c.c. | 0.0125 c.c. | 0.00625 c.c. | 0.004 c.c. | |
| 1 | T. O. | 21 | 27 | Corona | Inguinal | None | Pos. | Pos. | Pos. | Pos. | Pos. | Pos. | |
| 2 | H. C. | 21 | — | Lip | None | None | Pos. | Pos. | Pos. | Pos. | Neg. | — | |
| 3 | W. S. | 30 | — | Corona | Inguinal | None | Pos. | Pos. | Pos. | Pos. | Pos. | Neg. | |
| 4 | R. G. | — | 33 | Corona | Inguinal | None | Pos. | Pos. | Pos. | Pos. | Pos. | — | |
| 5 | H. L. | 31 | — | Corona | General | Papular | Pos. | Pos. | Pos. | Pos. | Pos. | — | |
| 6 | M. B. | 46 | — | Corona | Inguinal | Macular | Pos. | Pos. | Pos. | Pos. | Pos. | Weak pos. | |
| 7 | M. J. | 18 | — | Lip | General | Macular | Pos. | Pos. | Pos. | Pos. | Neg. | Neg. | |
| 8 | B.G. | 20 | — | Corona | Inguinal | Macular | Pos. | Pos. | Pos. | Pos. | Pos. | Pos. | |
| 9 | J. S. | 35 | 28 | Corona | Inguinal | None | Pos. | Pos. | Pos. | Pos. | Pos. | Neg. | |
| 10 | M. T. | 21 | 28 | Corona | Inguinal | None | Pos. | Pos. | Pos. | Pos. | Pos. | Weak pos. | |
| 11 | G. G. | 14 | — | Lip | General | None | Pos.? | Pos. | Pos. | Pos. | Pos. | Neg. | |
| 12 | B. B. | 21 | 28 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Pos. | Weak pos. | |
| 13 | J. S. | 10 | 28 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Pos. | Neg. | |
| 14 | W. F. | 14 | 24 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Weak pos. | — | |
| 15 | F. C. | 24 | 25 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Pos. | Neg. | |
| 16 | G. J. | 14 | 28 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Weak pos. | Neg. | |
| 17 | B. H. | 10 | 24 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Pos. | Neg. | Chancre treated with iodoform. |
| 18 | R. G. | — | — | Genitalia | General | None | Neg. | Pos. | Pos. | Pos. | Pos. | — | |
| 19 | E. J. | — | — | Corona | None | None | Neg. | Pos. | Pos. | Pos. | Pos. | — | |
| 20 | I. J. | 10 | 21 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Pos. | Neg. | |
| 21 | V. J. | 7 | — | Lip | General | None | Neg. | Pos. | Pos. | Pos. | Weak pos. | Neg. | |
| 22 | M. D. | 52 | — | Lip | Cervical | Macular | Neg. | Pos. | Pos. | Pos. | Neg. | Neg. | |
| 23 | T. M. | 14 | 24 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Pos. | Neg. | |
| 24 | H. L. | 6 | — | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Neg. | Neg. | |
| 25 | H. C. | 8 | — | Lip | Cervical | None | Neg. | Pos. | Pos. | Pos. | Pos. | Weak pos. | |
| 26 | S. A. | 3 | 28 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Neg. | Neg. | |
| 27 | B. W. | 10 | 21 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Neg. | Neg. | |
| 28 | M. C. | 4 | — | Lip | Cervical | None | Neg. | Pos. | Pos. | Pos. | Neg. | Neg. | |
| 29 | H. S. | 17 | 28 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Pos. | Neg. | |
| 30 | B. C. | 4 | 28 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Weak pos. | Neg. | |
| 31 | F. L. | 7 | 23 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Pos. | Neg. | |
| 32 | H. V. | 7 | 28 | Genitalia | None | None | Neg. | Pos. | Pos. | Pos. | Weak pos. | Neg. | |
| 33 | J. Y. | 4 | 28 | Corona | Inguinal | None | Neg. | Pos. | Pos. | Pos. | Weak pos. | Neg. | |
| 34 | A. T. | 5 | 21 | Lip | None | None | Neg. | Pos. | Pos. | Pos. | Neg. | Neg. | |
| 35 | J. Y. | 21 | — | Genitalia | None | None | Neg. | Neg. | Neg. | Neg. | Neg. | Neg. | No symptoms after 4 mos. Wass. neg. |
| 36 | G. H. | 10 | — | Genitalia | None | None | Neg. | Neg. | Neg. | Neg. | — | — | No symptoms after 3 mos. Wass. neg. |

*Days from first appearance of chancre.

**Days between first appearance of chancre and exposure.

TABLE II

SUMMARY OF RESULTS

| NO. CASES | LESION | BLOOD WASSERMANN | SECONDARIES | | DARK FIELD | | WASSERMANN ON CHANCRE SERA | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 0.1 cc. | | 0.0125 cc. | | 0.00625 cc. | | | 0.004 cc. | | |
| | | | Pres. | Abs. | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Pos. | Weak | Neg. | Pos. | Weak | Neg. |
| 10 | Primary chancre | Positive | 4 | 6 | 10 | 0 | 10 | 0 | 10 | 0 | 8 | 0 | 2 | 2 | 2 | 3 |
| 1 | Primary chancre | Weak pos. | - | 1 | 1 | - | 1 | - | 1 | - | 1 | - | - | - | - | 1 |
| 23 | Primary chancre | Negative | 1 | 22 | 23 | 0 | 23 | 0 | 23 | 0 | 11 | 6 | 6 | 0 | 2 | 18 |
| 2 | Chancroids | Negative | 0 | 2 | 0 | 2 | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 |
| 36 | | | 5 | 31 | 34 | 2 | 34 | 2 | 34 | 2 | 20 | 6 | 10 | 2 | 5 | 23 |

of primary syphilis. We do not in any sense suggest this reaction as a substitute for the dark-field, which should be employed in all cases. But in cases in which no dark-field microscope is available, in cases in which the dark-field examination is negative, and in cases, particularly, where treatment of a lesion with a spirocheticide has driven the spirochetes from the chancre, the local Wassermann reaction should be employed. In such circumstances the local Wassermann reaction is the only positive diagnostic procedure available earlier than the appearance of a positive blood reaction, which does not occur until there is systemic invasion. Because of the very great importance of a prompt diagnosis, this simple procedure has a definite place in the diagnosis of primary syphilis in the earlier stages.

The local Wassermann was positive in all of our thirty-four cases of demonstrated primary chancre, in the majority of which the blood Wassermann was negative and secondary manifestations were absent. A local as well as hematogenous origin of the specific complement-fixing antibodies appears definite. In all cases 0.0125 c.c. of the serum was sufficient to give the positive reaction. The simplicity with which such a small amount can be obtained from the surface of a chancre by diluting with several drops of saline, disposes of any suggestion of difficulty in securing sufficient serum to carry out the reaction. In a later communication we hope to report on the practicability of preserving and mailing tubes of serum to distant laboratories for the performance of the Wassermann test.

## CONCLUSIONS

1. The Wassermann reaction carried on the chancre sera of 34 demonstrated cases of primary chancre was positive in all cases.

2. Of the 34 cases giving positive local Wassermann reactions the majority showed negative blood reactions and no secondary manifestations.

3. The local Wassermann reaction was positive with 0.0125 c.c. in all cases and in some cases with only 0.004 c.c. of chancre serum.

4. Treatment of the lesions locally with a spirocheticide appears to have no effect upon the complement-fixing antibodies, although the spirochetes may disappear.

5. A local as well as hematogenous site of origin for the specific complement-fixing antibodies appears established.

6. In cases where no dark-field microscope is available, where the spirochetes are not found, or where a lesion has been treated by a spirocheticide, the local Wassermann reaction is a practicable, simple and valuable means of diagnosis of primary syphilis at the time most important for prognosis and treatment.

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# THE ACTION OF PROTEINS AND BLOOD SERUM ON COLLOIDAL GOLD SOLUTION AND ITS QUANTITATIVE INTERPRETATION*

By Paul Reznikoff, M.D., New York City

THE INTERPRETATION of the colloidal gold reaction has occupied the attention of investigators since its introduction. Although Lange believed the phenomenon to be one of protein chemistry, the effect of the individual proteins in cerebrospinal fluid upon the colloid, other views were entertained and experimental evidence was presented to support them. Scant attention need be paid to the inorganic elements of cerebrospinal fluid or blood serum. The concentration of inorganic electrolytes is negligible with respect to any action on colloidal gold solution. The consideration of ferments or specific antibodies as the factors in the production of the curves can have but little weight in view of the very definite and conclusive action of the proteins upon colloidal gold.

Zsigmondy introduced his work on colloidal solutions with the very significant statement that although Faraday preceded him by many years in his experiments on colloids, this was rather an incentive than a deterent. He pointed out that no research can be found which has not been touched upon by some former worker usually unknown to the new enthusiast. In this very field the problems which are still important and unsolved have been studied by the pioneers in colloidal chemistry. Lange, himself, used blood serum on colloidal gold solution and drew conclusions from his meagre results.

The exact action of the proteins upon colloidal solutions is more than of academic interest. Our methods for the isolation and study of these protein bodies are very unsatisfactory. The best determinations are essentially approximate. That the proteins play a very important part in our metabolic processes can be of very little doubt. Even rough estimates of the total and individual blood serum proteins point to marked differences in certain clinical conditions.

## PURE PROTEINS

With these considerations in view pure proteins† were tested against colloidal gold and definite curves obtained. These proteins,—serum albumin,

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†The euglobulin was prepared by precipitation from fresh serum with a saturated solution of sodium chloride. The pseudoglobulin was prepared by precipitating both the euglobulin and the pseudoglobulin by half saturation with ammonium sulphate and removal of the pseudoglobulin by washing with saturated sodium chloride. These steps were repeated several times. The resulting proteins contained the faintest trace of the other globulin according to a precipitin test, but they were found to be the purest globulins that could be obtained by separation with salts. Of course, we have no evidence that the globulins are distinct immunological entities and cannot rule out chemical purity by the precipitin test. The albumin was found to be free from other proteins.

serum pseudoglobulin and serum euglobulin, were obtained from Dr. Arthur F. Coca, of the Department of Immunology of the New York Hospital. Preliminary work was done with proteins secured from the Bureau of Laboratories, Department of Health, New York City, and certain commercial products. The pure albumin contained 3.36 mg. nitrogen per c.c., was in aqueous solution and could be tested directly against the colloid. The pseudoglobulin and euglobulin were not dialysed to be freed from the excess salt but were precipitated with 95 per cent alcohol, centrifuged, washed with 80 per cent alcohol three times, centrifuged each time, and then washed into a dish with 95 per cent alcohol. The mixture was dried by an electric fan and the globulins taken up with 0.4 per cent saline. The resulting solutions contained 0.448 mg. pseudoglobulin nitrogen per c.c. and 0.35 mg. euglobulin nitrogen per c.c. The colloidal gold solution was prepared according to the method of Lee. It was found that a constant solution, neutral to alizarin, was obtained which was very sensitive, normal cerebrospinal fluids giving a curve. The reason for this extreme sensitivity was probably the excess and complete addition of formaldehyde before heating; but this was found to be desirable because of the constancy of the various lots of solution. The details of cleaning the glassware were adhered to as carefully as for the regular colloidal gold test.

The albumin-colloidal gold reaction was tested by setting up twenty-nine tubes. In the first, 2 c.c. of the stock solution was placed, in the second, 1 c.c., in the third, 1 c.c., and in each of the remaining tubes 0.5 c.c. of 0.4 per cent saline. 0.5 c.c. of the albumin solution was removed from the third tube and put into the fourth. The saline and albumin were thoroughly mixed and 0.5 c.c. of the mixture was put into the fifth tube. This procedure was repeated thoughout all the tubes until the last, where the 0.5 c.c. of the mixture was discarded. The resulting series, therefore, started with an albumin nitrogen content of 6.72 mg. in the first tube, 3.36 mg. in the second, 1.68 mg. in the third, and so on. By diluting each succeeding tube with an equal quantity of saline as indicated above, the twenty-ninth tube contained 0.00000002 mg. of albumin nitrogen. Each tube thus contained half the quantity of albumin of the preceding tube. Only twenty-nine tubes were used because preliminary tests showed that after that no further reaction was obtained. Five c.c. of colloidal gold solution was added to each tube and the tube was thoroughly shaken. The first tube to decolorize completely was noted, marked "x" on the charts, and the reaction was read in twenty-four hours. In the reading, the same numbers were used to refer to the colors as in the regular Lange reaction, namely:

| | | | |
|---|---|---|---|
| Red | (0) | Blue | (3) |
| Red-blue | (1) | Light blue | (4) |
| Violet | (2) | Colorless | (5) |

The greater the precipitation of the colloidal gold, the higher the number reads. (5) represents complete precipitation.

The pseudoglobulin test involved the use of twenty-four tubes. Here, 3 c.c. of the pseudoglobulin solution was used in the first tube, giving 1.344

mg. nitrogen of this protein, 2 c.c. in the second, 1 c.c. in the third and fourth, and so on, as outlined in the albumin test. The dilutions were brought up to 0.5 c.c. with 0.4 per cent saline except for the first three tubes which contained 3 c.c., 2 c.c. and 1 c.c. respectively of the original 0.4 per cent saline solution of the pseudoglobulin. Here again, 5 c.c. of colloidal gold solution was added and readings made as for the albumin test.

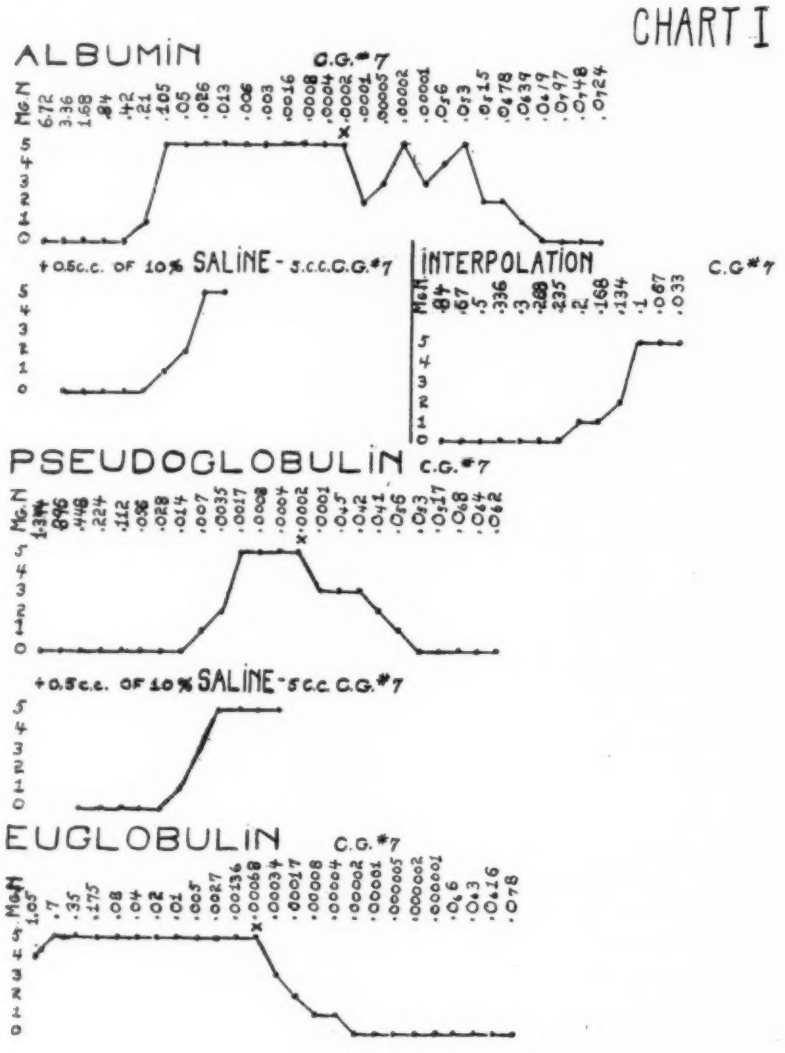


Chart I.

The euglobulin tubes numbered twenty-five. The first tube contained 3 c.c. of the euglobulin solution, containing 1.05 mg. nitrogen of this protein; the second containing 2 c.c., the third, 1 c.c., and so on, as indicated above, the dilutions of all the tubes except the first three being 0.5 c.c. Here again, the readings were made as for the two preceding proteins.

For albumin an interpolation test was performed through the phase

where the first decolorization appears. Instead of halving the amount of albumin in each succeeding tube, definite amounts were made up thus: 0.25 c.c., 0.2 c.c., 0.15 c.c., 0.1 c.c., 0.09 c.c., 0.08 c.c., 0.07 c.c., 0.06 c.c., 0.05 c.c., 0.04 c.c., 0.03 c.c., 0.02 c.c., 0.01 c.c. and the result was noted.

The results obtained are given in the accompanying Chart I. The curves are seen to be distinctive for each type of protein. In the curves the abscissae represent the extent of decolorization; the dots on the ordinates, the tubes. "C.G.#" indicates the lot of colloidal gold solution used and the number of milligrams of nitrogen of the protein for each point on the curve is represented above the curve.

The curve obtained for serum albumin shows the following characteristics: From 6.72 to 0.21 mg. no precipitation occurs; at 0.21 mg., the precipitation begins (1). 0.1 mg. of albumin nitrogen precipitates the 5 c.c. of colloidal solution completely, a colorless tube resulting. At 0.0002 mg. the decolorization ceases and the curve tends to zero. But two secondary rises develop, at 0.00002 mg. and 0.000003 mg. the curve going up to (5). At 0.00000019 mg. the color is again red and remains so throughout further dilutions. The tube to decolorize completely most rapidly is the sixteenth containing 0.0002 mg. of serum albumin nitrogen.

The serum pseudoglobulin curve shows no precipitation until 0.007 mg. nitrogen of the protein is reached. Decolorization occurs at 0.0017 mg. and after 0.0002 mg. precipitation diminishes so that at 0.000003 mg. the curve reaches the base line and remains flat on further dilution. The first tube to decolorize completely for this protein is 0.0002 mg. nitrogen.

Serum euglobulin does not protect the colloidal solution in the highest concentrations obtainable. The curve starts at (4) and at 0.7 mg. nitrogen precipitates the colloid completely. After 0.00068 mg. the curve sinks and at 0.00002 mg. reaches a red color, never rising upon further dilution. The first tube to decolorize completely in point of time is the one containing 0.00068 mg. of euglobulin nitrogen.

Besides the definite quantitative action upon colloidal gold of known amounts of the proteins, the curves of each have their own individual characteristics. Serum albumin begins to precipitate colloidal gold solution in relatively high concentration. The phase of transition from the beginning to complete precipitation is a short one, whereas the phase of complete precipitation is long. Finally there are two secondary rises which are constant.

Pseudoglobulin does not begin to precipitate colloidal gold until in rather dilute solution. The rise of the curve is rapid and the phase of complete precipitation is very short. The descent is gradual and there are no secondary rises.

Euglobulin is characterized by the absence of a protective phase in even the most concentrated solutions that can be obtained for use against colloidal gold solution. The phase of complete precipitation is a long one, but having reached zero, the curve remains flat.

All the proteins have one feature in common, namely, the tube with the least amount of protein to decolorize completely is the first one to decolorize

with respect to time. Then the decolorization continues to the left. This is seen most commonly in a paretic cerebrospinal fluid curve.

The protective mechanism of the greater concentrations of albumin and pseudoglobulin is a well-known colloidal phenomenon. It is seen in the prozone of a precipitin reaction. The secondary rises of albumin, or perhaps more correctly, the two descents before the final permanent descent of the curve is probably a type of prozone.

*Gold Number.*—In connection with this work it is of interest to consider the so-called gold number. Zsigmondy defined this as the number of milligrams of a colloid that protects 10 c.c. of colloidal gold solution from further change after 1 c.c. of a 10 per cent sodium chloride solution is added. In the case of these proteins 5 c.c. of colloidal gold and 0.5 c.c. of 10 per cent saline were used. The chart shows that for serum albumin the gold number lies somewhere between 0.2 and 0.026 mg. nitrogen for the particular colloidal gold solution used. For pseudoglobulin the gold number lies between 0.02 and 0.0035 mg. nitrogen. Euglobulin has no gold number, failing to protect the colloidal gold from the 10 per cent saline in any concentration. This could be readily surmised from the character of the curve. It is noteworthy that albumin and 10 per cent saline have a higher protective action on colloidal gold than when albumin is used alone. This is an illustration of the fact that the combined action of electrolytes may not be cumulative but entirely opposite in effect from their action when used individually.

*Protein Mixtures.*—Combinations of the proteins were next tested. The first mixture consisted of 1 mg. albumin nitrogen, 0.5 mg. of pseudoglobulin nitrogen and 0.5 mg. of euglobulin nitrogen in the first tube, half these amounts in the second and so on. The resulting curve on Chart II shows a marked resemblance to euglobulin in the failure of the high concentrations to protect and the position of the phase of decolorization in the curve. Furthermore, the total number of milligrams of nitrogen in tube twelve, the first to decolorize in point of time, is 0.0009, resembling euglobulin in this respect. The effect of albumin is seen in the slight drop of the thirteenth tube to (4).

A second combination was made from the stock aqueous and 0.4 per cent saline solutions containing 2.25 mg. albumin nitrogen, 2.5 mg. pseudoglobulin nitrogen and 1.75 mg. euglobulin nitrogen. This was in accordance with a verbal report of the relative amounts of these proteins in normal horse serum found in the Laboratories of the Board of Health. A mixture of 0.67 c.c. of the albumin, 5.58 c.c. of the pseudoglobulin and 5 c.c. of the euglobulin solutions gave the above results. Using half the above mixture in the first tube, one quarter, in the second and halving the amounts in succeeding tubes, the number of milligrams of nitrogen in the first tube was 1.125 albumin, 1.25 pseudoglobulin and 0.875 euglobulin.

The curve, shown on Chart III presents four features. (a) There is a failure to completely protect in the greatest concentration used. The curve starts at (1) instead of (0) showing the euglobulin effect. (b) There is a long continued complete precipitation phase, resembling in extent and posi-

tion, the albumin curve. (c) The first tube in time and lowest in concentration to completely precipitate contains a total of 0.00019 mg. of nitrogen, the amount giving the same result in the albumin and pseudoglobulin curves. (d) The albumin effect is seen still further in the secondary rise. It can be seen that this mixture is dominated by albumin. Pseudoglobulin, apparently, has little effect because of the very nature of its curve. If anything, it has

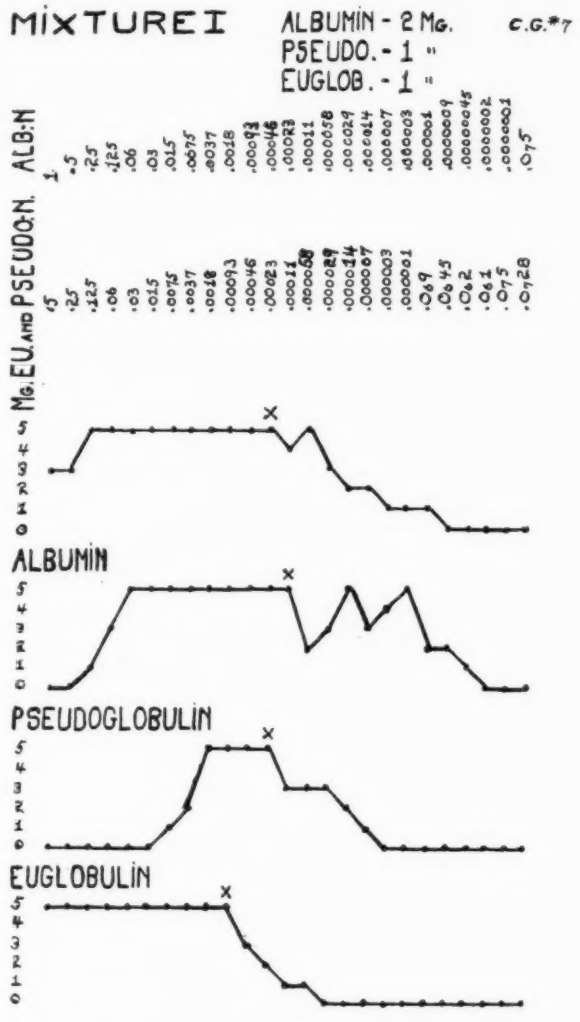


Chart II.

a protective action in the higher and lower concentrations, tending to overcome the effect of euglobulin in the higher concentrations and that of the secondary rises of albumin in the dilute mixtures.

*Blood Serum.*—Before undertaking the study of the effect of blood serum on colloidal gold solution, deproteinized serum was tested. Lange thought that there were no differences between the various blood sera curves, but when these sera were deproteinized he thought that luetic sera always showed much

stronger curves than normal. He used colloidal iron and dilute acetic acid as deproteinizing agents. It is difficult to understand how Lange could come to such conclusions and at the same time claim that the colloidal gold reaction was due to proteins. The explanation lies in the failure to use properly deproteinizing agents. Those commonly used either do not completely deproteinize or act upon colloidal gold themselves.

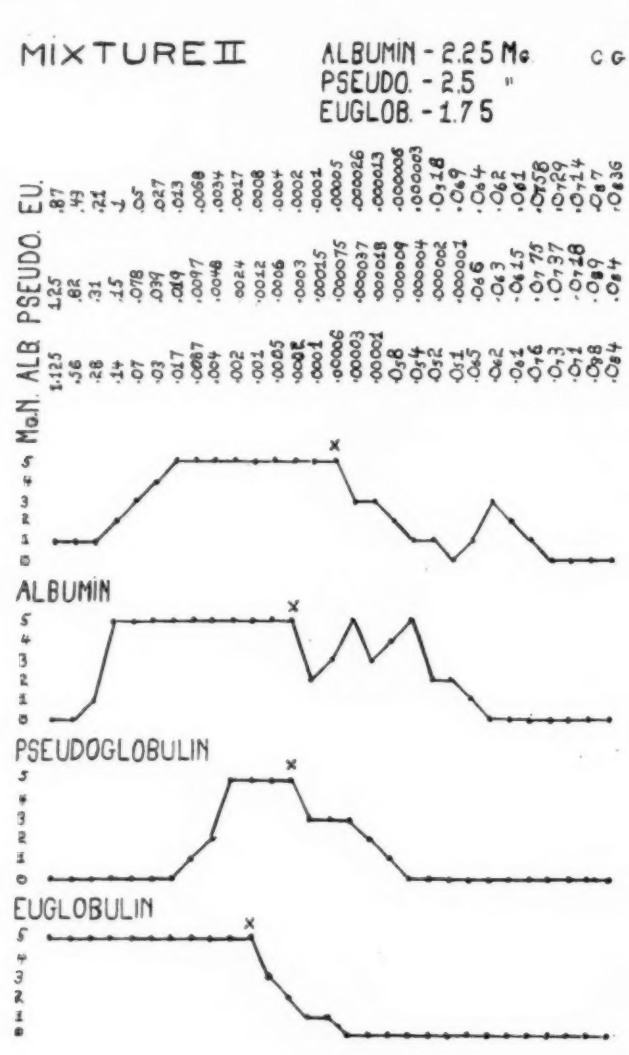


Chart III.

The following is a list of deproteinizing agents and their limitations for use in this work.

1. Acetic acid N/100 + heat – filtrate still gives Millon's and Biuret reactions.

2. Kaolin + acetic acid – filtrate precipitates colloidal gold slightly.

3. Phosphotungstic acid – filtrate is cloudy and gives Millon's reaction.

4. Tungstic acid precipitates colloidal gold completely.
5. Trichloracetic acid precipitates colloidal gold completely.
6. Colloidal iron – filtrate is colored.

The only agent that precipitated protein completely, so that the filtrate when evaporated to dryness gave no Biuret or Millon's test, and which itself neither precipitated nor protected the gold solution was alcohol. Ninety-five per cent alcohol was used in the proportion of 9 c.c. to 1 of serum and after thorough mixing was permitted to stand for twenty-four hours and filtered. The serum used in all cases was free from the slightest visible hemolysis.

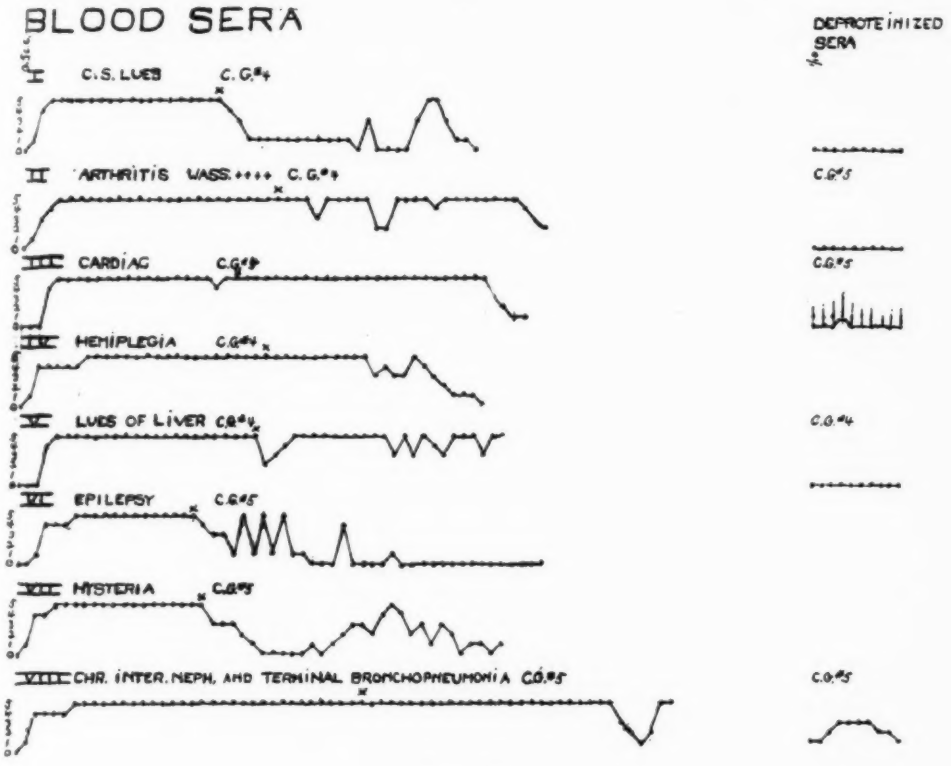


Chart IV.

On the right hand side of Chart IV, V, VI the results of deproteinized sera and colloidal gold are given. One c.c. of the 1-10 alcoholic filtrate was placed in the first two tubes. Five-tenths c.c. of 0.4 per cent saline was added to eight remaining tubes. Five-tenths c.c. of the alcoholic filtrate was removed from the second tube to the third and this process was continued through the remaining tubes so that the resulting tests represented 1/10 c.c. of the original serum in the first tube, 1/20 c.c., in the second and so on, the tenth tube being equivalent to 1/5120 c.c. of the serum which had been deproteinized. It can be seen that in one case the curve goes up to 1 and in two cases to 2 (Cases III, VIII, XI). In all the other cases the curves are flat indicating no protein. The slight rises in the curves men-

tioned indicate only the faintest traces of protein when the extreme sensitivity of the colloidal gold solution used is considered. Furthermore, in these three cases, as will be pointed out later, the curves of the original serum showed a marked increase in protein content. A rise of 2 with ordinary colloidal gold solution is considered high normal in testing cerebrospinal fluid. It must be remembered that the colloidal gold used in these experiments was of such delicacy that it gave a positive curve as high as 5 with normal cerebrospinal fluid. It may then be considered that serum deproteinized by alcohol has no action on colloidal gold solution except in those sera with marked increase of protein where apparently the slightest traces remain.

The sera from eight patients were taken, all at the same time of day. These cases were chosen at random to see the effects of the serum on colloidal gold solution. The tests were performed as for the pure proteins. One c.c. of the serum was placed in the first tube, 0.5 c.c. of 0.4 per cent saline in each

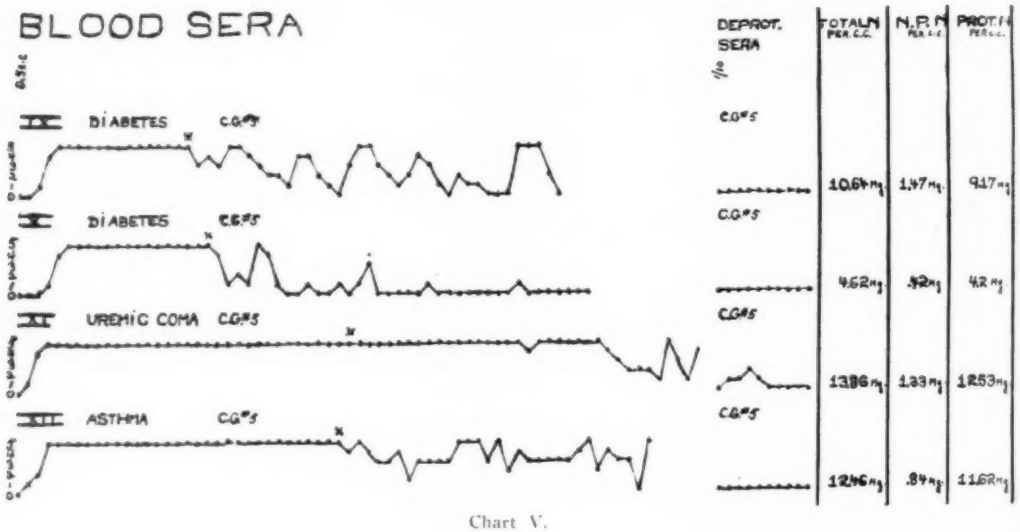


Chart V.

of the remaining tubes, as a rule sixty-one tubes being used. Five-tenths c.c. of the serum was transferred from the first tube to the second and so on; so that 0.5 c.c. of the original serum was contained in the first tube, 0.25 c.c., in the second, etc. All the dilutions were made up to 0.5 c.c. with 0.4 per cent saline. Five c.c. of the colloidal solution was added to each tube. Chart IV shows the marked variation of curves obtained. Practically no two curves resembled each other except for general appearance,—such as Cases I and VII. Case VIII was one of terminal chronic interstitial nephritis and bronchopneumonia and represents an extreme type of curve.

The factors involved in these results are: (a) the total amount of protein, (b) the amounts of individual proteins, (c) the combinations of individual proteins in each dilution. Therefore, a simple analysis and explanation of a curve is hardly possible because of the very nature of its complexity.

To obtain some idea of at least one of these factors, four cases were taken to determine the serum curve, the total nitrogen, the nonprotein nitrogen

in the alcoholic filtrate and thus the total protein nitrogen. Chart V shows the relationship of the curve to the total protein nitrogen. The protein nitrogen varied from 4.2 mg. per c.c. to 12.53 mg. per c.c. The higher the protein content, the more sustained the curve, that is, the more tubes completely decolorized. Curve XI is a case of uremic coma and shows the most marked effect of the protein. Curve X, a diabetic of long standing shows the least effect. It can readily be seen that the nonprotein nitrogen has no effect, case IX having a serum nonprotein nitrogen of 1.47 mg. per c.c. and case XI of 1.33 mg. The total protein content had, therefore, a marked effect on the "intensity" of the curve.

The other factors involved in the production of a colloidal gold curve with serum are not easily determined. There is no chemical method for determining proteins which is accurate and practical. Howe's sodium sul-

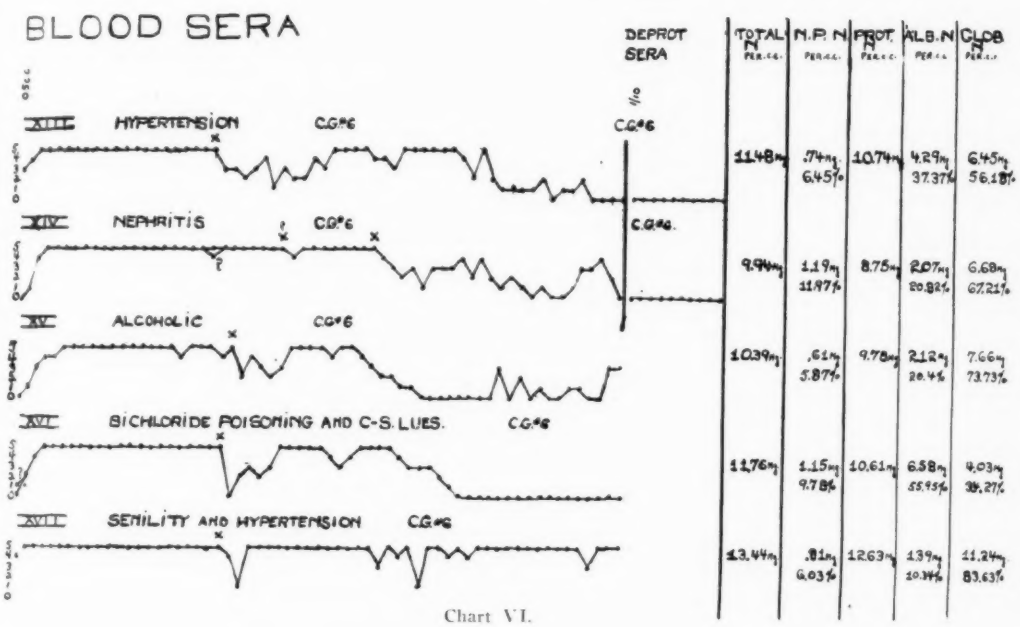


Chart VI.

phate method was tested against the pure proteins to determine its applicability to these experiments. It was found that neither 14 per cent nor 22.2 per cent sodium sulphate had any effect on the albumin; that the 14 per cent salt precipitated 97.88 per cent of the euglobulin; that the 22.2 per cent salt precipitated 96.04 per cent pseudoglobulin; but that the 14 per cent solution precipitated 89.16 per cent of the pseudoglobulin, thus making the determination of the separate globulins impossible.

An analysis of the curves shown on Chart VI cannot be made in great detail. In general, the intensity of curve depends on the total protein content, —curve XVII showing this most markedly with 12.63 mg. of protein nitrogen per c.c. of serum. The proportion of albumin in the sera was found to be lower than is usually given, varying from 10.34 per cent to 55.95 per cent of the total nitrogen. Curve XIII in all probability indicates a large amount

of euglobulin, by the failure of the curve to start at 0, but beginning at 3. Curve XVI shows best two distinct secondary rises and would thus indicate relatively more albumin. This is actually the case, 55.95 per cent of the total nitrogen belonging to that protein. Curve XVII shows 83.63 per cent globulin nitrogen, and indicates much euglobulin, the start taking place at 4.

No real explanation of the effect of serum on colloidal gold can be made, however, until simple and accurate chemical methods for separating and determining the proteins are found. The importance of obtaining such methods can be seen in the great variation of total and individual protein content of the sera in the different clinical conditions indicated on the charts. Probably more proteins play a part in the production of the curve than we can now isolate and determine. The great variation in total protein content of blood sera is in itself a subject that has been given little attention and may be of considerable importance.

While these tests were in progress, Heinrich Fischer published an account of his elaborate and detailed experiments on the mechanism of the colloidal gold reaction in cerebrospinal fluid. His methods involved separation of proteins by means of ammonium sulphate and preparation of his colloidal gold with the aid of glucose. His results show that the curves obtained were due to the action of proteins. In one finding there is wide disagreement from the results obtained in this work. He finds albumin has no effect on colloidal gold, but actually protects the solution from other proteins and electrolytes. In the experiments described in this paper albumin was found to have a very distinct and characteristic curve.

## SUMMARY AND CONCLUSIONS

1. Solutions of pure serum albumin, serum euglobulin and serum pseudoglobulin were tested against colloidal gold solution and were found to have definite and characteristic curves for each protein.

2. These curves depend upon the concentration of the protein in solution that acts as a protecting or precipitating agent of the colloidal gold.

3. A gold number was obtained for the serum albumin and serum pseudoglobulin, with the colloidal gold solution used in these tests.

4. Mixtures of the three proteins were tested with the colloidal gold solution and an analysis of the curves obtained showed the influence of the individual proteins depending on their concentrations in the mixtures and upon their normal action.

5. (a) Blood serum can be deproteinized with 95 per cent alcohol and the filtrate obtained will have no effect on colloidal gold or in extreme cases a negligible effect.

(b) The colloidal gold reaction is a protein phenomenon.

6. Human sera show marked differences in their effect on colloidal gold solution in various clinical conditions.

7. The total protein content of a serum is an important factor in maintaining a curve. The "intensity" of the curve varies directly with the amount of protein present.

8. In various clinical conditions the amount of protein nitrogen and the percentages of albumin and globulin found in sera vary greatly.

9. The curves are influenced by the variations in albumin-globulin ratio.

10. Howe's method for isolating the globulins was not found to be accurate by testing it with the pure proteins used in these experiments, the euglobulin precipitating agent precipitating 89.16 per cent of the pseudoglobulin.

11. The results obtained with serum albumin show it to have a distinct precipitating effect on colloidal gold in certain definite concentrations, thus differing from the findings of Fischer.

12. New methods for the separation and determination of proteins are necessary before the very obvious and probably important differences of these substances in various clinical conditions can be investigated.

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# THE CENTRAL INFLUENCE OF ATROPINE AND HYOSCINE ON THE HEART RATE*

By W. J. R. Heinekamp, Chicago, Ill.

RECENTLY McGuigan[1] reported that pharmacopoeial doses of atropine, 0.0005 gm., cause slowing of the heart only, when given to adults. This he believed to be the result of central stimulation. Large doses, however, paralyze the vagus endings almost immediately so that the central action of atropine is unable to influence the heart. Harris[2] confirmed McGuigan's findings and states that doses of 0.00065 gm. in man were not strong enough to effect paralysis of the vagus endings, but that the slowing which occurred was evidently due to vagus stimulation. Paskind,[3] in a series of experiments, found that while a small dose of atropine causes slowing of the heart in white adults, as a rule it produces acceleration in negroes.

Sollmann[4] states that atropine stimulates the vagus center in common with other medullary centers and hence may cause a primary slowing. Cushny[5] states that "even therapeutic doses slow the pulse for a short time apparently from direct action on the heart." Also, that the action of atropine on the vagus endings is purely depressant (loc. cit. p. 12). Bush[6] using 0.02 per cent atropine found that it exerted little or no influence on the cardio-inhibitory center of the turtle.

Hyoscine, according to Cushny[7] does not alter the pulse, except that it may be slower, owing to the hypnotic action. Sollmann states that the pulse may be unchanged, or considerably slowed from central vagus stimulation.[8] I have found that atropine in small doses—0.0003 gm. when injected subcutaneously in the dog produces slowing of the heart. This action is not manifested after section of the vagi or with anesthesia, and hence is central. I have also found that atropine and hyoscine produce inhibition of the heart when perfused through the medulla of the yellow bellied terrapin (Pseudomys troosti). Hyoscine, however, also depresses the cardio-inhibitory center.

## EXPERIMENTS

### SERIES A

The methods used in perfusing the medulla were those reported in a previous article.[9] Atropine sulphate (Merck) and hyoscine hydrobromide (Merck) were used, dilutions being made up from 1 per cent solution.

The following experiments illustrate the various results obtained; namely:

1. Inhibition produced by atropine.
2. Inhibition produced by hyoscine.
3. Inactivity of strychnine and atropine after hyoscine.

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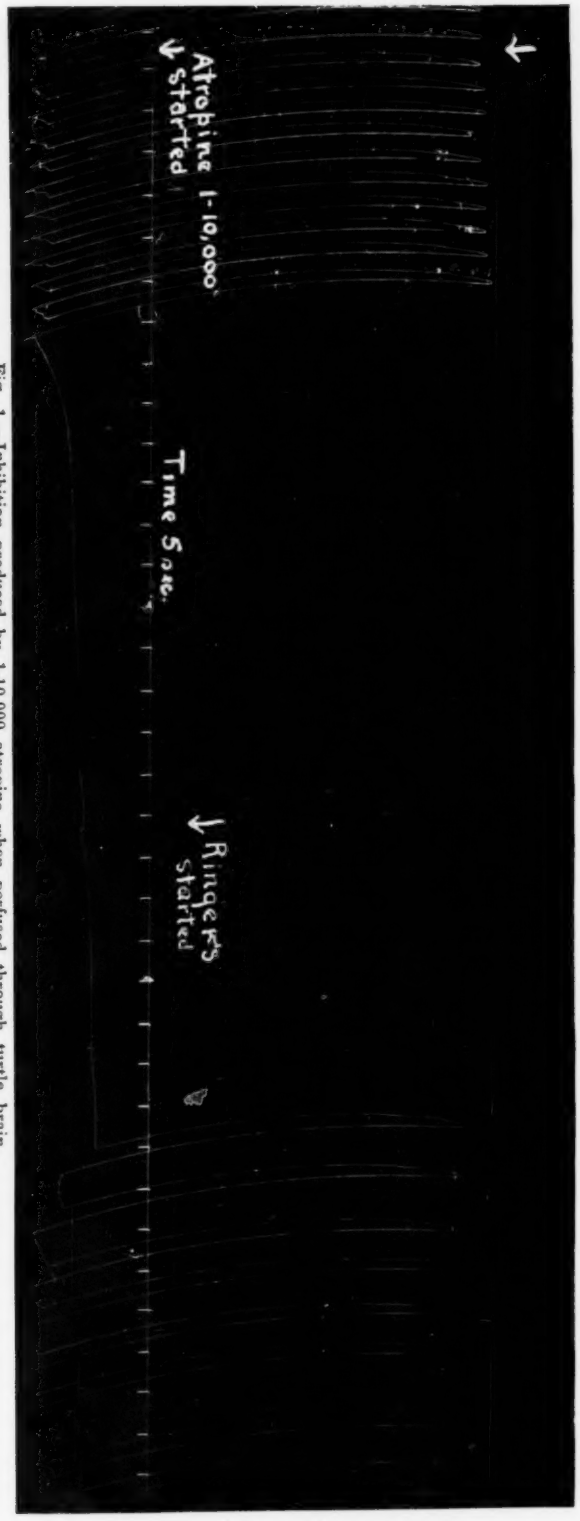


Fig. 1.—Inhibition produced by 1-10,000 atropine when perfused through turtle brain.

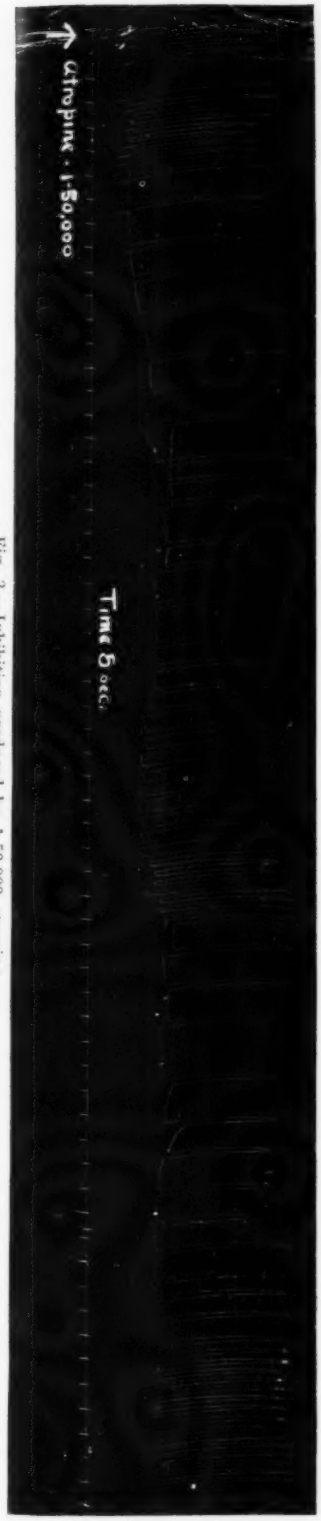


Fig. 2.—Inhibition produced by 1-50,000 atropine.

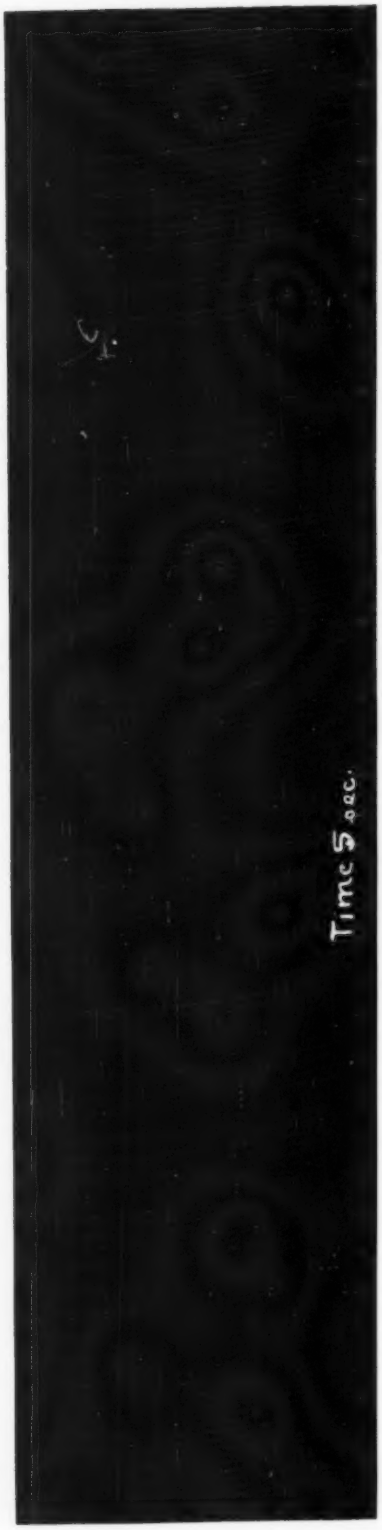


Fig. 3.—Inhibition produced by 1-100,000 atropine.

Fig. 4.—Inhibition produced by 1-10,000 hyoscine.

EXPERIMENT 1.

| Time (min.) | Remarks | Rate |
|---|---|---|
| – | Normal | 34 |
| 9 2/3 | After perfusion with 0.02 per cent atropine sulphate. | 6 |
| – | After perfusion with Ringer's | 38 |
| 1/4 | After perfusion with 0.01 per cent atropine sulphate. | 8 |
| – | After perfusion with Ringer's | 36 |
| 1/2 | After perfusion with 0.002 per cent atropine sulphate. Slowing persisted 12 minutes. | 8 |
| – | After perfusion with Ringer's | 39 |
| 3 | After perfusion with 0.001 per cent atropine sulphate. | 10 |
| – | After perfusion with Ringer's | 37 |

EXPERIMENT 2.

| Time (min.) | Remarks | Rate |
|---|---|---|
| – | Normal | 20 |
| 1/2 | After perfusion with 0.01 per cent atropine sulphate. | 0 |
| – | After perfusion with Ringer's | 26 |
| 2/3 | After perfusion with 0.01 per cent atropine sulphate. | 0 |
| – | After perfusion with Ringer's | 25 |
| 4 | After perfusion with 0.002 per cent atropine sulphate. | 24 |
| 1 | After perfusion with 0.01 per cent atropine sulphate. | 0 |
| – | After perfusion with Ringer's | 25 |
| 1 | After perfusion with 0.01 per cent atropine sulphate. | 0 |
| – | After perfusion with Ringer's | 28 |
| 2 1/2 | After perfusion with 0.01 per cent hyoscine hydrobromide. Slowing persisted 11 minutes | 0 |
| – | After perfusion with Ringer's | 22 |
| 5 | After perfusion with 0.01 per cent atropine sulphate. | 24 |

EXPERIMENT 3.

| Time (min.) | Remarks | Rate |
|---|---|---|
| – | Normal | 48 |
| *2 | After perfusion with 0.01 per cent hyoscine hydrobromide | 8 |
| – | After perfusion with Ringer's | 42 |
| 2 | After perfusion with 0.01 per cent strychnine sulphate | 10 |
| – | After perfusion with Ringer's | 50 |
| – | After perfusion with 0.01 per cent hyoscine hydrobromide | Irreg. |
| 10 | After perfusion with 0.01 per cent strychnine sulphate | 46 |
| 14 | After perfusion with 0.01 per cent atropine sulphate. | 44 |

*Heart had started to return to normal before Ringer's was used.

SERIES B

In this series well-nourished dogs were injected subcutaneously with atropine sulphate in olive oil. The slow absorption kept the concentration such that while the cardio-inhibitory center was stimulated, the vagus endings were not paralyzed. When atropine was injected in a water solution, absorption occurred so rapidly that the slowing phase was either materially

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shortened or did not occur at all. Inhibition did not occur in any experiment after section of the vagi, or while the animal was under the influence of ether anesthesia. It was noted that after the slowing phase the heart became very irritable and irregular, due to the mixture of central stimulation and peripheral depression. This was more marked when a water solution of atropine was used than when olive oil was used as a menstruum. Fig. 5 shows two typical experiments.

It is very necessary to keep the animal quiet throughout the entire ex-

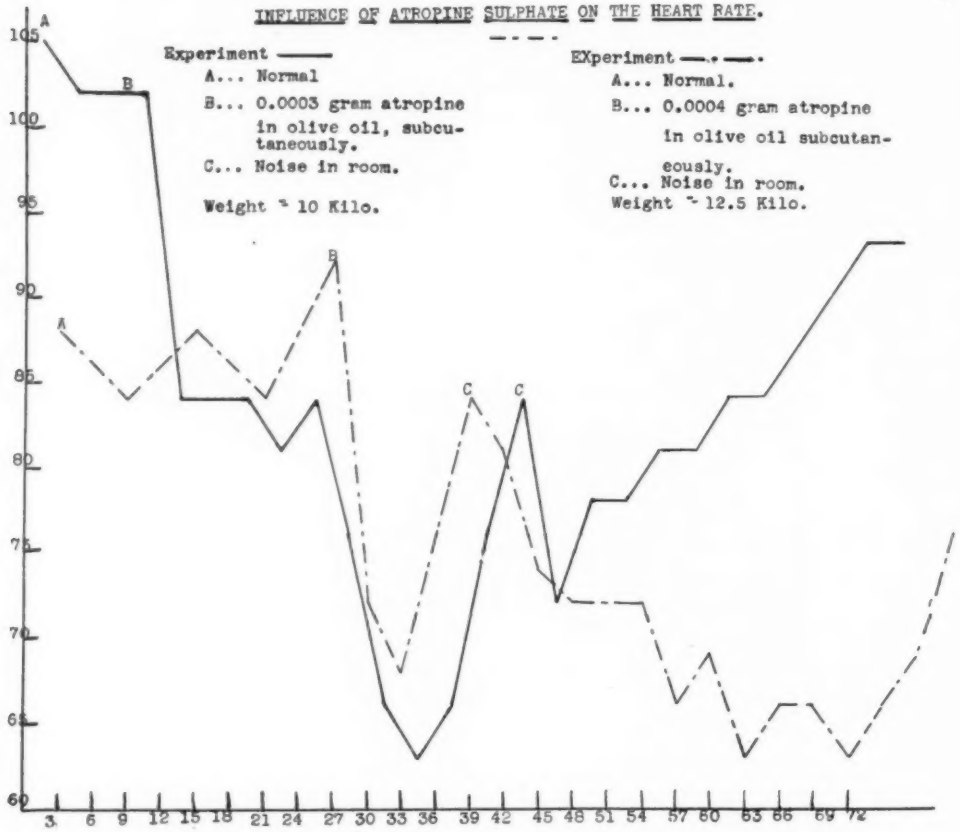


Fig. 5.—Chart showing influence of atropine sulphate on the heart rate of dogs.

periment since even slight noise causes a rapid rise in pulse rate, especially when atropine begins to depress the vagus terminations.

Fig. 6 is from the work of Dr. McGuigan. The patient was a well adult man, and was given 0.0005 gm. of atropine sulphate by mouth. Slow absorption probably accounts for gradual prolonged slowing.

Fig. 7 is from the work of Dr. Paskind, of this laboratory, and illustrates the difference in susceptibility to atropine which the white and negro race exhibit. Both subjects received 0.001 gm. of atropine sulphate subcutaneously. Negroes are relatively unsusceptible to the central action of atropine.

## DISCUSSION

Analysis of the results obtained in the above experiments shows that atropine sulphate in small doses causes inhibition of the heart. While this may be due to:

1. Stimulation of the cardio-inhibitory center.
2. Stimulation of the intrinsic ganglia of the heart.
3. Stimulation of either the parasympathetic or sympathetic nerve endings to the heart.
4. Direct action on the heart muscle; I believe that it is due to direct stimulation of the medulla.

Atropine exerts no influence on ganglion cells, except in massive doses when they are depressed along with all cells. After the injection of an amount of atropine sufficient to produce mydriasis, stimulation of the cervi-

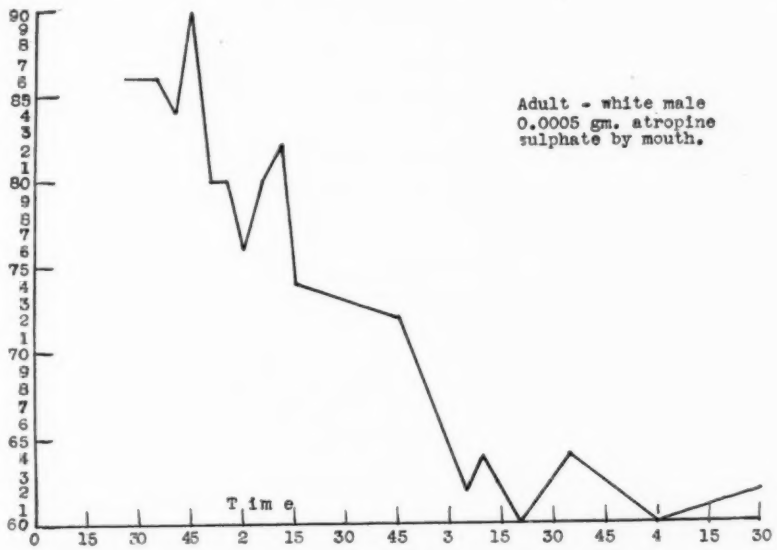


Fig. 6.—Chart showing the influence of atropine sulphate on the heart rate of an adult white and of an adult negro when given subcutaneously.

eal sympathetic nerve below the superior cervical ganglion still produces a further dilation of the iris, also constriction of the ear vessels, and a flow of saliva. These facts indicate clearly that atropine has no influence on ganglion cells, and hence the probability of atropine producing inhibition by stimulating the intrinsic ganglion of the heart, is excluded.

The influence of atropine directly on heart muscle is negligible. Gruber and Markel[11] state that atropine increases the general tonus of the terrapin heart muscle, but that it exerts only a slight effect on the tonus waves, increasing them if anything. That the slowing after the injection of atropine is not due to its action on the myocardium is proved by lack of inhibition after section of the vagi.

The peripheral action of atropine is characterized by depression of the parasympathetic myo-neural junctions. It exerts no influence on the sym-

pathetic endings to the heart. In my work on dogs I have found that after section of the vagus, or when an animal is under anesthesia, atropine will not produce inhibition.

The inhibition effected by atropine is due to stimulation of the cardioinhibitory center. This is supported by the following facts. When atropine is perfused through the medulla of the yellow bellied turtle, inhibition of the heart results. Since the two vagi are the only connections between the medulla and the heart in these experiments, the slowing must be due to the influence of atropine on the brain. This is not due to cerebral inhibition since atropine stimulates the central nervous system. It is due, therefore, to direct stimulation of the cardioinhibitory center of the medulla.

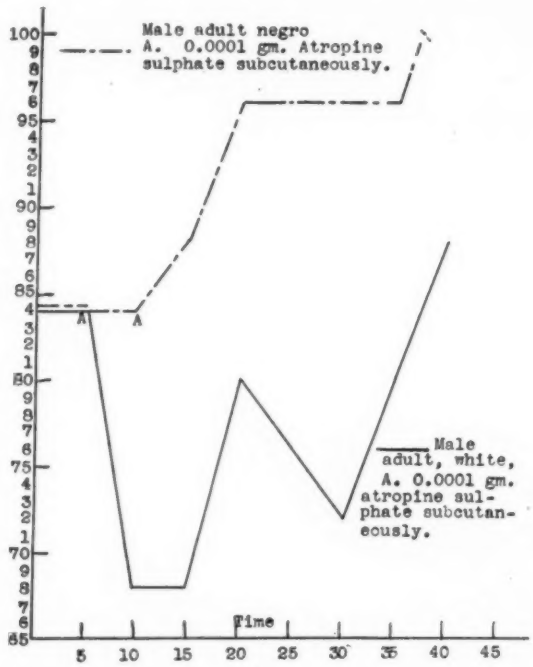


Fig. 7.—Chart showing influence of atropine sulphate on an adult white when given by mouth.

In the dog no inhibition occurs after section of the vagi so that the action is limited to the central nervous system. Likewise, during ether anesthesia no inhibition results. This is probably due to the depression of the cardioinhibitory center by the anesthetic, atropine, paralyzing the vagus endings before it is able to stimulate the depressed center, as stated by McGuigan.

The action of atropine on the heart rate is determined by the amount of drug used. Apparently the strong drug paralyzes the center for when compared on a time basis, I have found a 0.01 per cent solution to produce inhibition in the turtle more promptly than a 0.02 per cent solution. A 0.002 per cent solution produces a more lasting inhibition, but not so marked or so prompt as a 0.01 per cent. A 0.001 per cent solution is weakly effective, but if perfused for a sufficient length of time may produce inhibition.

Likewise, when large doses of atropine are injected into a dog, inhibi-

tion is generally not produced since the paralysis of the vagus endings prohibits the influence of central stimulation. However, if the atropine sulphate be suspended in olive oil, the absorption is so retarded that the concentration of atropine necessary to stimulate the center is attained, but the amount necessary to paralyze the vagus endings is not reached.

These experimental findings corroborate the results of McGuigan and Harris both of whom obtained clinical evidence that atropine in small doses, 0.001 gm., produces slowing of the heart. With larger doses, acceleration results, due to peripheral paralysis of the vagus.

The action of hyoscine on the turtle brain is similar to that of atropine. Hyoscine, however, depresses the center subsequently, strychnine which I found previously[11] and which Bush[12] later confirmed to be a powerful stimulant of the cardio-inhibitory center, or atropine failing to cause slowing after hyoscine has been used.

## CONCLUSIONS

Small doses of atropine—0.0003 gram in dogs, 0.001 gram in man produce inhibition of the heart due to direct stimulation of the cardio-inhibitory center.

Hyoscine exerts a similar action to that of atropine on the medulla of Pseudomys troosti except that the primary stimulation is followed by depression.

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[3]Paskind: Jour. Lab. and Clin. Med., 1921, vii, 104.
[4]Sollmann: Textbook of Pharmacology, 1917, ed. 2, p. 280.
[5]Cushny: Textbook of Pharmacology, ed. 7, 328, 21.
[6]Bush: Jour. Pharmacol. and Exper. Therap., 1919-20, xiv, 313.
[7]Cushny: Textbook of Pharmacology, ed. 7, p. 331.
[8]Sollmann: Manual of Pharmacology, p. 298.
[9]Heinekamp: Jour. Pharm. and Exper. Therap., 1922, xix, 131.
[10]Gruber and Markel: Ibid., 1918, xii, 53.
[11]Heinekamp: Ibid., 1919, xiv, 17.
[12]Bush: Ibid., 1919, xiv, 313.

# BASAL METABOLISM AND BLOOD SUGAR TOLERANCE*

By George Howard Hoxie, M.D., F.A.C.P., Kansas City, Missouri

THE object of these studies was to determine the clinical value of the methods described; and also to ascertain the relation, if any, between the basal metabolism (thyroid activity) and the carbohydrate tolerance. For the literature on these matters had emanated from research laboratories and large hospital clinics, and it seemed necessary to have a trial of the methods under conditions of actual practice.

The material for these studies consisted of 95 consecutive patients whose condition suggested a study, either of the carbohydrate tolerance or of the basal metabolic rate. Of these patients, eleven were studied more than once. Consequently, we have a list of 106 tests on 95 patients.

The basal metabolic rate was tested on a closed circuit type of apparatus, namely, the Sanborn Benedict, and the Sanborn Handy.

The amount of glucose used was 1.75 grams per kilogram of body weight, according to the suggestions of Janney.[1] The blood was taken at the end of one-half hour, at the end of one hour, and at the end of two hours. The dose of glucose based on body weight seemed more rational than the fixed dose of 100 grams for all weights and sizes of patients, and the two-hour period seemed long enough to determine the type of curve.

The blood was drawn with a 2 c.c. syringe and introduced into a test tube containing a small amount of sodium fluoride and gently shaken to prevent coagulation. The first specimen was taken with the patient in a basal state, 12 to 15 hours after the last food. A specimen of urine was also collected; and then the standardized glucose mixture was given. The second specimens of blood and urine were taken again at the end of the first half hour; the third specimens at the end of the hour after the glucose meal had been given. And the fourth specimens were taken at the end of the second hour. The technic for the determination of blood sugar was the one used with Epstein's Micro-Saccharimeter, as follows:

Two-tenths of a cubic centimeter of the blood is introduced into a standardized tube; then the tube is rinsed with water and diluted up to the 1 c.c. mark. Saturated picric acid is then added—a few drops at a time—up to the 2.5 c.c. mark, shaking the tube gently with each addition of acid. This is then allowed to stand a few minutes, and the contents centrifuged. One c.c. of the supernatant centrifuged fluid is put in a test tube and heated carefully until complete evaporation has taken place. Care is taken not to char the residue. Five-tenths cubic centimeter of a 10 per cent solution of sodium

*Received for publication, July 7, 1922.

[1]Janney, N. W., and Isaacson, V. I. I.: The Blood Sugar in Thyroid and Other Endocrine Diseases, Arch. Int. Med., 1918, xxii, 160.

carbonate is introduced into this tube and gently heated. This is then transferred to the graduated tube used in a small colorimeter and compared with the two standard Tubes A. and B. The solution in the graduated tube is diluted until it matches that in one of the graduated tubes, and the calculation made therefrom.

### GLYCOSURIA

We found 22 patients who showed sugar in their urine under the conditions of this test. The point at which the sugar appeared is shown on the

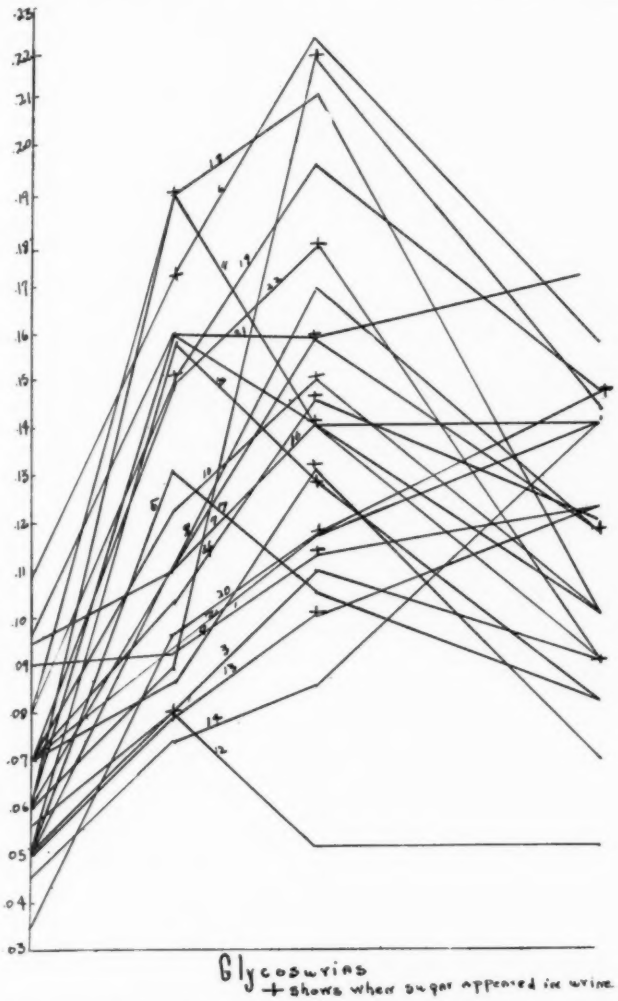


Fig. 1.—Curves of patients whose urine showed sugar during the test. A cross indicates the time at which the sugar was found.

accompanying chart (Fig. 1) by the "+." This, of course, indicates that the sugar appeared at some point between the two last determinations and should not indicate that the threshold for that individual for that particular amount of glucose was to be found exactly at the point where the "+" is placed. Of

this group, only one had been called a glycosuriac before this test, and in that case, the glycosuria was an accompaniment of hyperthyroidism of the adenomatous type (where a preceding colloid goiter had suddenly become active).

Of these 22 glycosuriacs, eight showed a basal metabolic rate more than 10 per cent above normal. One showed a minus 13 per cent, the rest (13) were within normal limits.

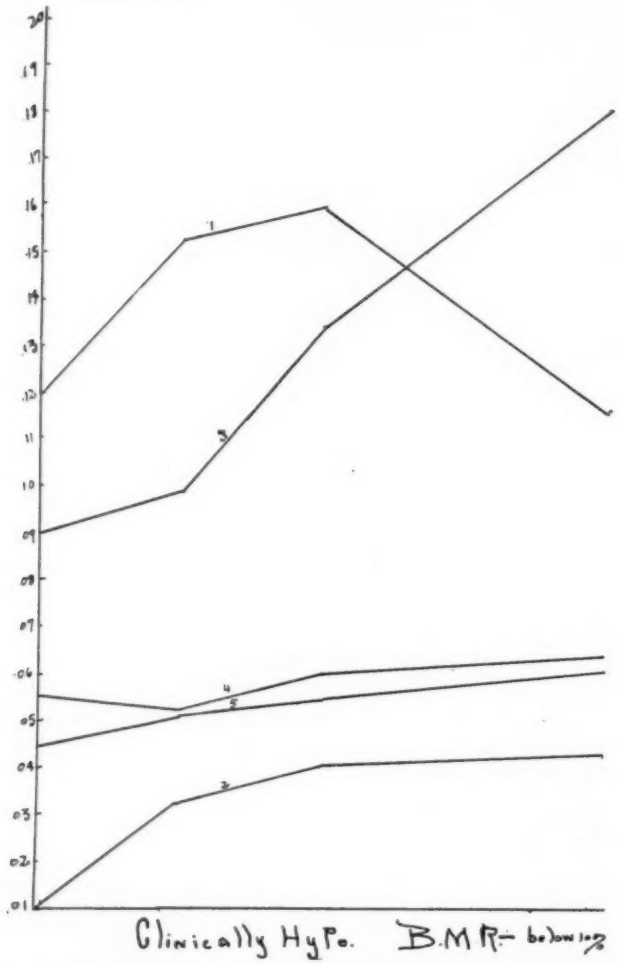


Fig. 2.—Curves of cases in which the calorimeter showed a BMR more than 10 per cent minus.

If we accept as the normal curve for sugar tolerance the return at two hours to approximately the fasting point, and the diabetic curve where the percentage of blood sugar is still increasing at the end of two hours, we find in this group seven diabetic curves. We find only three strictly normal curves; the others show a delayed return to the normal.

The threshold for the appearance of the sugar in the urine is below twelve in six cases. It is interesting to note that only four of the six show the typical diabetic curves; the other two (12 and 9) show the normal curve.

We find, therefore, glycuresis for this amount of glucose in two types of curves: first, those of slow rise and delayed return with the glycuresis occurring below the accepted threshold of 16.5, and, secondly, in those with a quick rise and that to a height above 16.5.

*Hypothyroid Cases. I.*—There were five cases (Fig. 2) in which the basal metabolic rate was more than 10 per cent below normal and in which the clinical symptoms were sufficient to justify a diagnosis of thyroid insuffi-

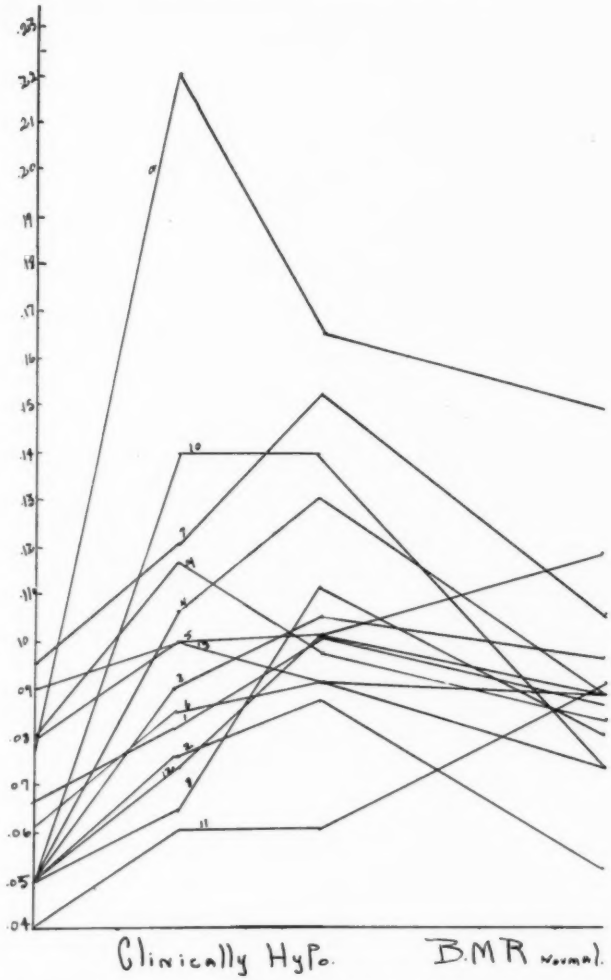


Fig. 3.—Curves of patients with normal calorimetric readings, but where the clinical working hypothesis had been hypothyroidism.

ciency; that is, where there was an agreement between the clinical and laboratory finding of hypothyroidism. It is interesting to note that four of these five cases show typical diabetic curves, but the fifth shows a normal curve in spite of the fact that the fasting sugar percentage is 0.12. One of these cases was studied three times; and after the first study thyroid extract was given. Ten months later, the basal metabolic rate was still slightly below normal but the sugar content of the blood was higher, although the curve

was of the same type. Then the third study three months later was made in which the original low blood sugar content was found again.

*Hypothyroid Cases. II.*—Hypothyroid cases with evidence of plurigland-ular involvement. We have three curves, the basal metabolism of all three being lower than minus 10, (–11, –12, –13). The sugar curve in two of them is of the decided diabetic type; and the third is one of delayed return. This group would go toward supporting belief in the parallelism between the

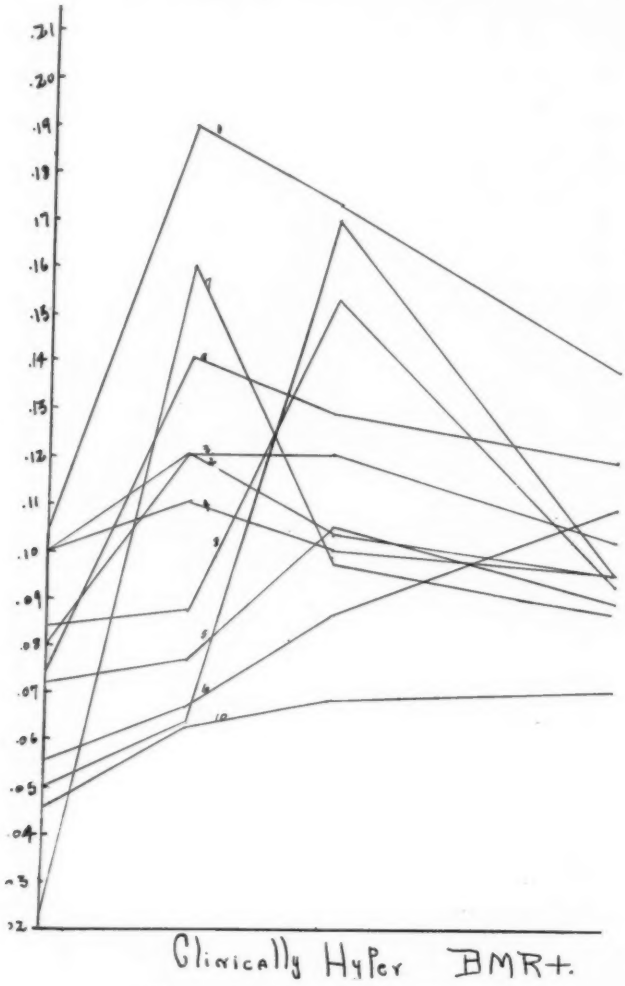


Fig. 4.—Curves in hyperthyroidism.

minus BMR and the diabetic curve. But the unknown factors in these cases were so great that such a conclusion would not be justified.

*Hypothyroid Cases. III.*—Senile Type. Here with a BMR reckoned variously at minus 9 and minus 14, we have practically a normal curve.

*Hypothyroid Cases. IV.*—Focal infections. We have three cases where there seemed to be a disturbance of the thyroid function, the basis for which, however, seemed to lie in focal infection elsewhere in the body. The first of

these, a recurrent stomatitis of the aspergillus type, showed a practically normal sugar curve. The second with a hepatic trouble, having a history of an operation for gall bladder, showed a BRM varying from –5.5 to –11 with a curve almost of the diabetic type. The third with a series of skin infections somewhat more virulent than an ordinary furunculosis with a BMR varying from –13 to –6 showed a curve of the delayed return type.

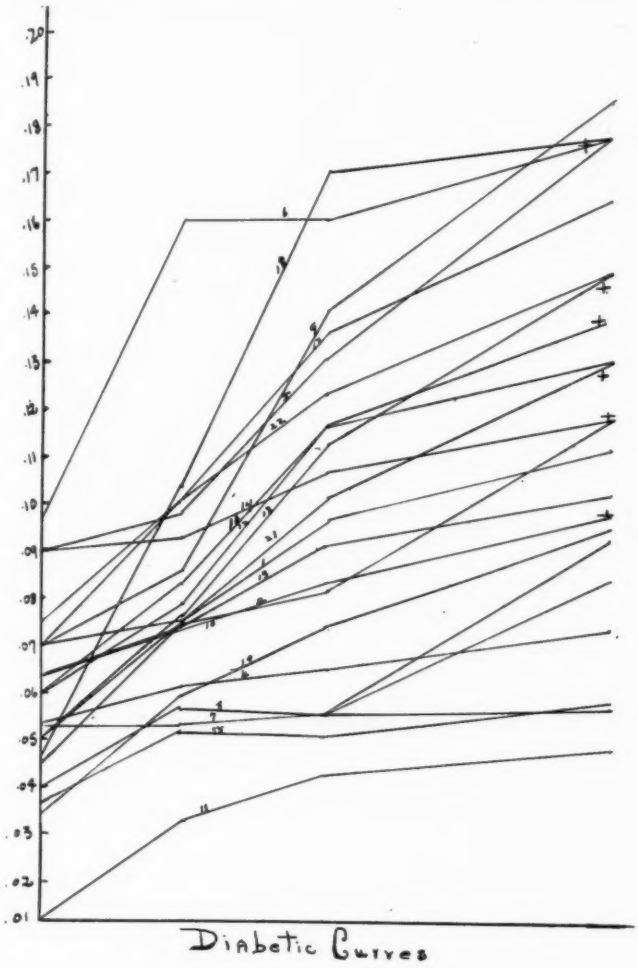


Fig. 5.—"Diabetic" curves.

*Cases clinically hypothyroid, but which showed a BMR within 10 per cent of the normal.*—Fourteen patients came in this class and the sugar curves (Fig. 3) showed two of the so-called diabetic type, four with delayed return, the rest being practically normal. It is interesting to note also that the one case where the sugar curve did not approximate those of the rest of the general group was in a patient where the suspicion is raised that instead of hypothyroidism, the patient may be developing pernicious anemia.

*BMR Normal.*—We had twenty-nine patients whose BMR was within normal limits. In this group, we find one case of an obesity with a –9; two cases

of epilepsy, one –4 and the other –2; several cases of exhaustion, etc. The curves for the most part are within normal limits, but the few exceptions deserve attention. Of the diabetic curves, one was a case of asthma; another was a case of chronic ulcerative colitis; another was a neurasthenic who would not obey any rules of hygiene; still another was a case of incipient tuberculosis.

*Hyperthyroidism. First*: Cases in which both the BMR was above 10 per cent and there was clinical evidence of the same condition. In this

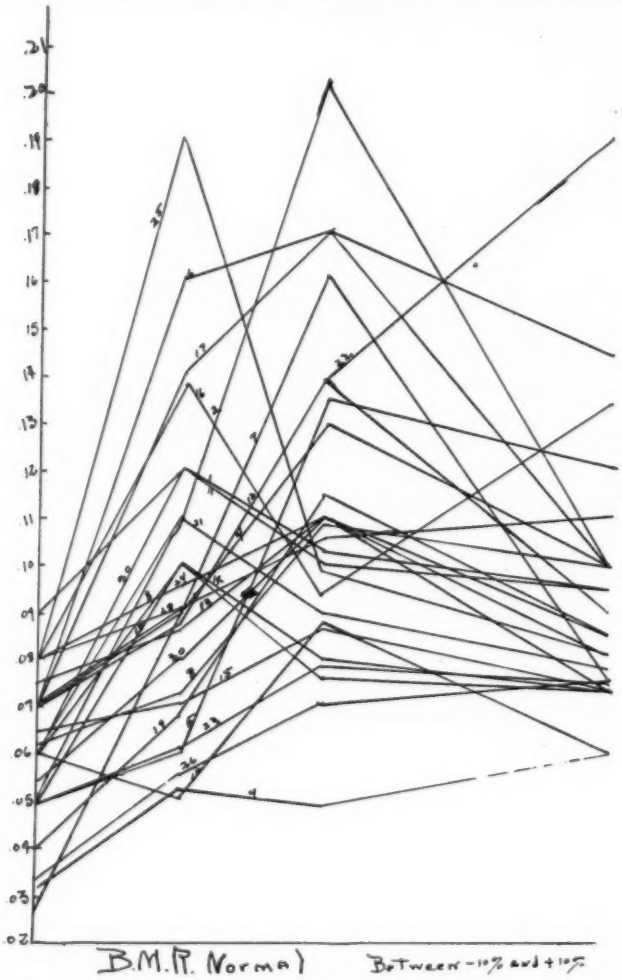


Fig. 6.—Graphs of sugar tolerance in cases wherein the calorimetric reading was within normal limits.

group, there were ten patients whose basal metabolism ran from plus 10 to plus 107. The sugar curves are less uniform than in the groups previously considered (Fig. 4). There were two distinctly diabetic curves, and in both these patients there is clinical evidence in favor of a pluriglandular syndrome.

Thus, a case (No. 7) of pure hyperthyroidism (adenoma) starts in with a fasting percentage of 0.023 and reaches in one-half hour 0.16. This curve is probably as nearly typical of pure hyperthyroidism as any of them; although curve No. 5 is that of another proved case of hyperthyroidism (in which an

operation was performed). The curve is of the same type except for the slow absorption. In this case, the curve starts with 0.05 and reaches 0.17 at the end of one hour. Curve No. 2 was that of a patient treated successfully with x-ray. Here two readings were taken at intervals of a month; and the interesting thing about it is that sugar is taken very slowly into the blood stream. For example, on August 20, 1920, when the BMR was plus 53, the sugar curves were .057, .062, .07, .075. On September 9, the BMR was plus 48,

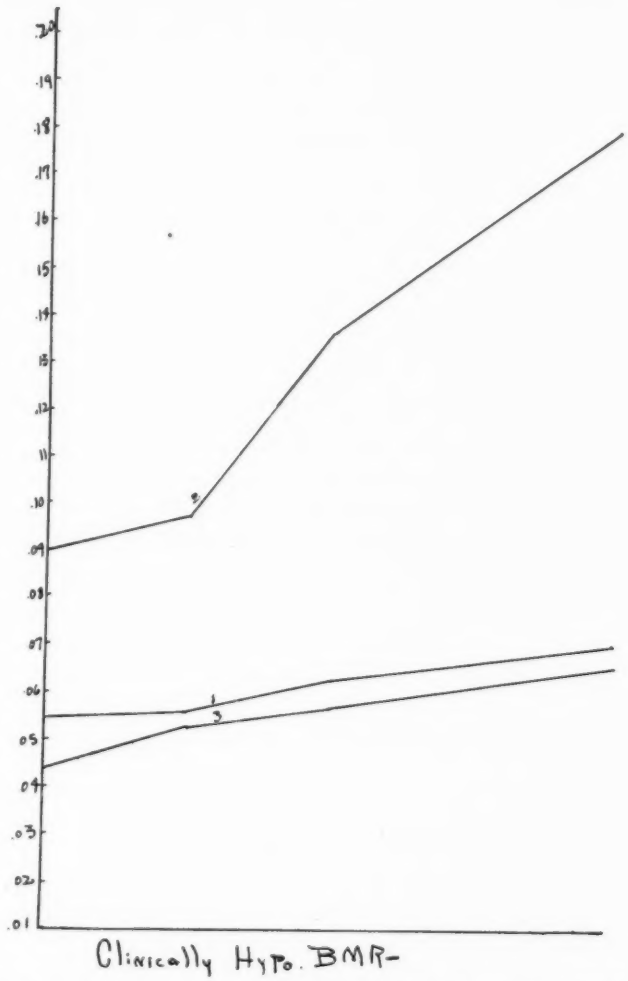


Fig. 7.—Curves of the sugar tolerance in cases wherein the calorimetric reading was also below normal.

and the blood showed .10, .12, .104, .10. It is evident in this case that the condition was not one of severe toxemia in spite of the fact that there was some myocarditis present, for which the patient came originally for treatment.

*Second.*—Cases in which the BMR was above 10 per cent but where the cases clinically seemed to be more neurotic than goitrous. In this group we have six curves, and as might be expected no uniformity. One curve is

extremely low, indicating a hypoglycemia. Another is strictly a diabetic curve. And another is the type with the slow return to normal with a long plateau, such as was marked up by Janney as being due to the loss of the thyroid. In our case, however, there was no evidence of loss of thyroid function either clinically or with a calorimeter.

*Third.*—Cases in adolescents. Of the adolescent group, we have three curves. One patient came in on account of his heart; and his curve shows the sharp rise and the equally sharp fall, while the BMR was plus 22. The second was a boy who was rather dull at school. His curve is nearly normal except that the rise is very slow. This patient was improved by the administration of thyroid extract. The third case was a girl who represented clinically the typically adolescent patient with a prominent thyroid gland (colloid goiter).

## "DIABETIC" CURVES

We have plotted out nineteen curves wherein the direction at the end of the second hour was still upward (Fig. 5). These fall into a general group with the exception of three curves. The first of these exceptions—where there is a hypoglycemia—has to do with a post-influenzal condition with a minus 20 BMR and considerable pulmonary distress with occasional fever. Here the readings were .01, .032, .04, .045. Another is that of a patient with a history of headache since the birth of her last child, at that time two years of age. The headache was constant but worse when rising from the horizontal position. The blood pressure was exceedingly low but the BMR was within normal limits. The readings were .097, .16, .16, .18. The renal threshold was low and sugar appeared in the urine. The third was a case of exhaustion with the symptoms of duodenal ulcer. Here the BMR was plus 15 and the renal threshold normal. The readings were .047, 0.103, .17, .18.

A most interesting comment on the so-called diabetic curve is that of all of these twenty-two cases showing the typical form, only six showed sugar in the urine during the test.

## DELAYED SUGAR ABSORPTION

We have four cases of delayed absorption where the angle of the curve at the half hour interval is less than 160 degrees. Three of these show a minus BMR; one shows glycosuria with extreme hyperglycemia. The fourth curve in this group is marked up with a plus BMR, but I believe that that was due to the patient's not being in a basal state. Unfortunately, the test was not repeated.

These results tend toward substantiating the statement often made that delayed absorption of the glucose accompanies decreased metabolism.

## CONCLUSIONS

1. Our experience would lead us to believe that the simple closed circuit types of calorimeters are worth while in actual practice.

2. The variations in reports on the BMR seem due more to the condition of the patient than to the type of apparatus.

3. Such reports, however, are not final. It is necessary to correlate clinical observation with the laboratory tests.

4. The blood sugar tolerance test cannot be substituted for the calorimeter, nor any close parallelism drawn. Both studies are needed if a complete knowledge of the patient's function and vitality is sought.

5. A revision of the terminology and standards of the blood sugar curves is needed if we are to introduce this study into general practice.

# *LABORATORY METHODS*

## A SIMPLE METHOD TO DETERMINE THE COAGULATION TIME OF THE BLOOD*

By Henry J. Goeckel, Phm.D., Cranford, N. J.

EVERY one who has checked surgical cases, especially those to be operated on for nose and throat affections, by determining the coagulation time of the blood, knows the value of this test. It detects cases liable to post-operative hemorrhages if the coagulation time is not materially reduced by appropriate therapeutic means. It enables the surgeon or the attending physician to anticipate trouble and to prevent it or to be prepared to check a hemorrhage should it occur.

On a busy hospital service to employ the Buffi-Brooks or the Brodie-Russell-Boggs coagulometers is time consuming, while the testing of a liberal sized drop of blood on a plain glass slide is open to criticism because the margin of error is greater than is allowable.

The method employed by the writer possesses several advantages for hospital routine use. The equipment is very simple, inexpensive, and is economical in time. This last factor is always an important one in a busy hospital. The blood is not exposed in a large air space and is not liable to desiccation as in other methods.

### TECHNIC

The finger tip or ear lobe is thoroughly cleaned with alcohol or ether, rubbing it quite briskly with a clean piece of cloth, gauze or cotton to remove as much lymph as it is possible to remove from the area to be pricked, and at the same time to produce a somewhat hyperemic condition.

Three by one inch glass slides, each with two ground-out cavities are used. These are the stock slides used for hanging drop cultures and for the microscopic Widal agglutination tests for typhoid bacillus infections, etc. The cavities are of about fifteen millimeters' diameter and approximately matched.

The time of the appearance of the drop of blood after making the puncture is carefully noted and a rather liberal long drawn drop of blood is placed in each of the cavities of one slide by inverting the cavities over the puncture. This slide is then placed on top of the second slide as is shown in the illustration. This gives enclosed chambers of small size, each with a drop of blood suspended on the upper wall of the chamber. It is not neces-

*Received for publication, May 3, 1922.

sary to vaseline the slides to make them air tight if they are matched, neither is it necessary to clip them together if one is reasonably careful.

When the slides are tilted on ends as illustrated, the non-coagulated blood has a tendency to gather toward the bottom border giving a contrast of a light and a dark zone, which effect is accentuated by the slight magnification due to the curved surface of the cavity. This tilting is repeated about every thirty seconds after the third minute, alternating the incline to cause the blood to flow toward the lighter area. When the coagulation begins, a distinct clumping of the cells becomes visible. When the coagulation is completed no mobility will be seen, that is all parts of the drop will become fixed or coagulated.

By this method a coagulation time up to seven minutes appears to be a safe limit.

Since employing this method at Muhlenberg Hospital we have had two hemorrhage cases. One was a known bleeder anaphylactic to serum, who had received lactates without success (thyroid deficient), and another case which by oversight was not tested. This case showed a postoperation coagulation time of eleven minutes.

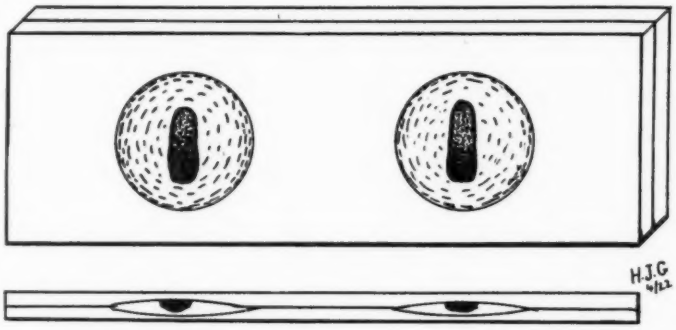


Fig. 1.

## SUMMARY

The advantages for this method are:

The equipment is simple and inexpensive.

By using a fresh slide for each case, many tests can be made in rapid succession without having to stop to clean the surface of an apparatus each time.

Each blood is tested in duplicate.

There being no air currents or large air space, evaporation is reduced to a minimum.

The curved surface of the cavity being somewhat magnifying, no lens is necessary to observe the changes.

The used slides can all be thoroughly cleaned at one time saving time and material.

The method is satisfactory for routine use. If anything it is somewhat more accurate than the special methods mentioned and is much more accurate than the exposed drop method.

# A MASK FOR THE ALVEOLAR AIR TESTING OUTFIT*

By Henry J. Goeckel, Phm.D., Cranford, N. J.

FOLLOWING is a description and illustration of a mask used with the Marriott Alveolar Air Testing Outfit used for the rapid clinical determination of carbon dioxide tension. It was improvised by the writer from material usually at hand in an average hospital.

The material required is an inflatable rubber cushion ring such as is used on the face mask of the Flagg or other anesthesia outfit for gas inhalation; a piece of rubberized cloth from a discarded hot water bottle or a piece of heavy rubber sheeting; a piece of glass tubing flared at one end and tapered somewhat at the other end; a strip of adhesive plaster or rubber

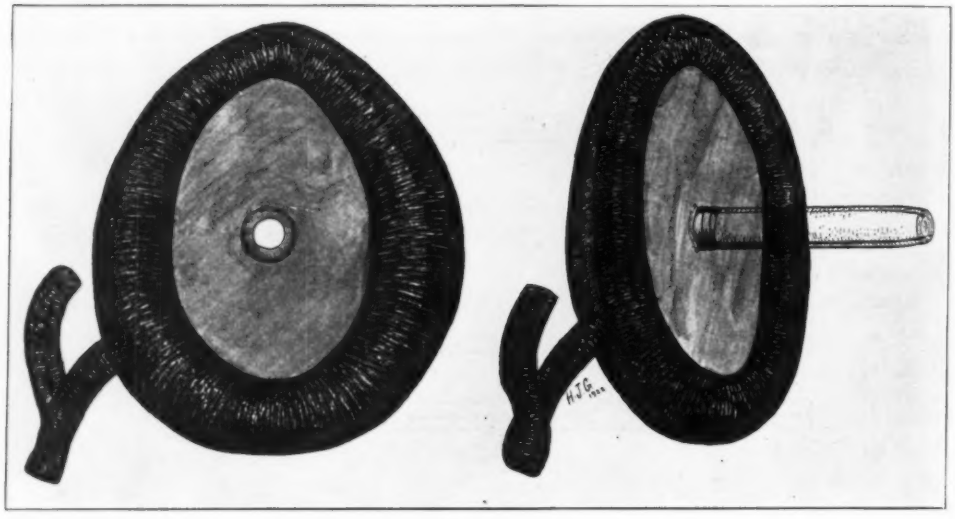

Fig. 1.

tissue; and a suitable rubber cement. The illustration shows how these are put together.

The cushion is well inflated and the side rubber tube is closed with the aid of an elastic band. This gives a masked breathing tip which can be securely placed over the nose and mouth of the patient and can be held firmly in place by the operator without elaborate preliminary adjustments and without discomfort for the patient. The air bag is attached to the glass tip in the usual manner.

Requiring practically no cooperation by the patient except to breathe, it can be employed equally well on conscious and on comatose cases. Being comparatively simple in appearance and comfortable when applied, it is not likely to arouse apprehension in the patient preceding the test. It possesses the important advantage that it can be readily cleaned.

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# A SIMPLE APPARATUS FOR CONTINUOUS EXTRACTION OF LIQUIDS BY LIQUIDS LIGHTER THAN WATER*

By Floyd De Eds, San Francisco, Cal.

THE quantitative extraction of liquids with ether and other solvents lighter than water by means of a separatory funnel is tedious, inaccurate and impractical. This is true of such a fluid as urine which tends to emulsify readily with ethereal solvents and, therefore, quantitative recovery is greatly impaired; and also of large volumes of fluids containing small quantities of solutes. Similar conditions arose in this laboratory in connection with a study of the urinary excretion of volatile and non-volatile salicyl ester compounds. For this purpose an apparatus that could be operated automatically, yet accurately, with large volumes of urine without attention was required. None was available in the market. Therefore, the apparatus described in this paper was devised and found to answer our requirements. It is believed that it is suitable for a variety of fluids and ethereal solvents. The advantages of this apparatus are simplicity, ease of construction and availability and satisfactory operation for quantitative purposes.

The principle employed in this apparatus is continuous, slow percolation of ether through the fluid to be extracted in an ordinary drug percolator. The supernatant layer of ether is automatically drained by an outflow tube which can be set at any desired level, depending on the volume of fluid (urine, etc.), to be extracted. A small, glass stop-cock is attached to the outflow tube for withdrawal of the ether extract to determine completion of the extraction. The percolation tube consists of two tubes and a receiving end for a condenser, all in one piece. One tube has a perforated bulb and descends into the liquid in the percolator and the other is outside the percolator for returning ether from the extraction flask to the condenser, which is attached to the receiving end of the tube. A diagram of the apparatus is presented in the accompanying figure and requires no explanation.

The apparatus is so simple that it can be easily constructed in the laboratory from parts available in the market. The following parts and their dimensions are used in the construction of an apparatus of 500 c.c. capacity: One pint drug percolator with large cork stopper to fit upper end snugly; outflow tube made of glass tubing 3 mm. bore and 31 cm. long from top to bend at neck of percolator, with a small thistle-like enlargement at the upper end 8 mm. diameter. This enlargement facilitates drainage of the ether extract and should be small enough to pass through the rubber stopper in the neck of the percolator. The lower end of the tube is bent at right angles

*From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco. Received for publication, June 6, 1922.

at the neck of the percolator and again at the extraction flask, the distance between angles being about 6 cm. Between the angles an ordinary burette stop-cock is fused for withdrawal of ether for testing. The tubing used for the percolation tube is of 1 cm. bore, the straight tube immersed in the percolator being 30 cm. long from the receiving end to the bulb and the bent outside tube being 45 cm. long from the angle and 3 cm. from the angle to the receiving end, which has a cork stopper with one hole for a short Liebig condenser. An extraction flask of 150 c.c. capacity is used. This is joined to the outflow tube and the outside portion of the percolation tube through a 2-hole cork stopper. For an apparatus of smaller capacity the parts are correspondingly reduced in size. In order to avoid use of a large cork in the percolator, this part of the apparatus could be made from large tubing which is constricted to a narrow neck at the upper end. However, I have

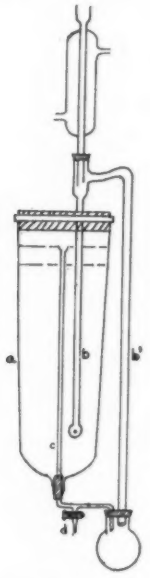


Fig. 1.—Apparatus for extraction of liquids by liquids lighter than water. *a*, percolator; *b*, percolation tube with outside tube, *b'*; *c*, outflow tube with stop-cock, *d*.

experienced no difficulties with percolators, which have the additional advantages of being easily available and cleaned and possessing considerable strength. Leakage of volatile substances through the cork stoppers is completely avoided by applying 15 per cent gelatin over their surfaces and allowing it to harden.

*Operation.*—With the outflow tube in position, the percolator is clamped to an iron stand and the fluid to be extracted is poured in. Then the percolating tube is adjusted to the percolator and extraction flask and the solvent is allowed to flow gently through the percolating tube until about 25 c.c. flow over from the supernatant layer into the extraction flask. Then the Liebig condenser is attached and the extraction flask is placed on the surface of water in an ordinary wash-pan maintained at about 64° C. by a thermoregulator, using an electric coil immersed in the pan or an ordinary electric

plate for heating. A shallow sand-bath placed over an electric hot plate is also convenient. In this way a battery of 6 to 12 extractions can be operated automatically and continuously without attention. In adjusting the outflow and percolating tubes the following points should be borne in mind: (1) With a given volume of liquid to be extracted, the length of the overflow tube must be such that the open end of the tube is far enough above the surface of the liquid in the percolator to allow for any increase in volume due to solubility of the solvent. With 500 c.c. of urine the increase in volume after continuous percolation with ether from 48 to 58 hours amounts to about 5 per cent (25 c.c.); (2) The percolating tube must be long enough to permit a certain amount of adjustment upward or downward so as to obtain the hydrostatic pressure necessary for passage of the solvent through the holes in the bulb.

An apparatus of 500 c.c. capacity has been tested several times with small quantities of salicylosalicylic acid and acetylsalicylic acid (nonvolatile) and methyl salicylate (volatile) added to 500 c.c. of urine. Table I indicates that the quantitative recovery is entirely satisfactory:

TABLE I

| VOLUME OF URINE USED (C.C.) | COMPOUND AND QUANTITY ADDED (GM.) | QUANTITY RECOVERED (GM.) | PER CENT RECOVERY | DURATION OF EXTRACTION WITH ETHER (HRS.) |
|---|---|---|---|---|
| 500 | Methyl salicylate 0.10 | 0.09 | 99 | 41 |
| 500 | Salicylosalicylic Acid 0.2 | 0.21 | 98 | 58 |
| 500 | Acetylsalicylic Acid 0.010 | 0.0094 | 94 | 55 |

## CONCLUSION

A simple, convenient apparatus* for the continuous, automatic and quantitative extraction of nonvolatile (salicylosalicylic acid and acetylsalicylic acid) and volatile (methyl salicylate) solutes in liquids (urine) by liquids lighter than water (ether) is described.

Thanks are due to Dr. Hanzlik for suggestions in construction of the apparatus.

*The apparatus of different capacities, or any of its parts, is obtainable from Braun-Knecht-Heimann Co., San Francisco, Calif.

# MODIFIED ELECTRODE FOR USE IN CLINICAL ELECTROCARDIOGRAPHY*

By H. M. Korns, M.D., and E. J. Warnick, Cleveland, Ohio

AFTER more than a year's experience with the electrode described by Cohn,† we have found it advisable to introduce a minor modification. Adventure of salt into the crevices between washer, screw, and binding-post, and corrosion of these points of contact gives rise to annoying polarization currents and very high resistance. To assure uniform results it is necessary to remove the binding-post and renew all contact surfaces immediately before making each record. This difficulty has been obviated by changing

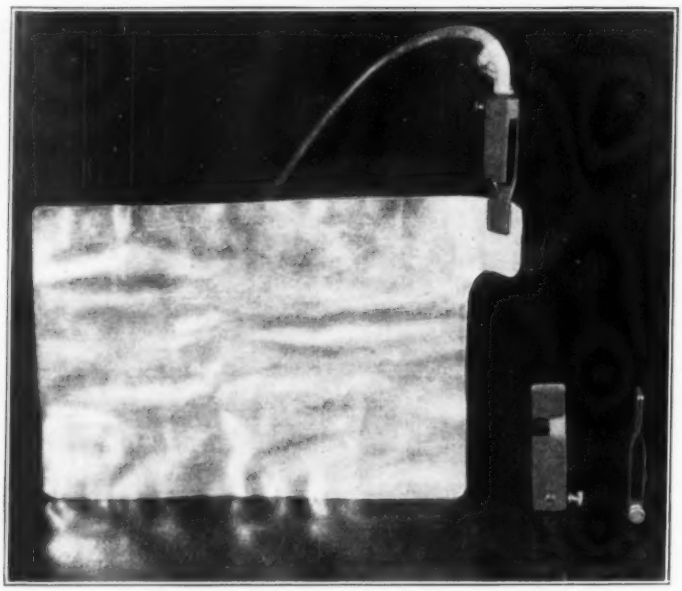

Fig. 1.

the method of making contact. To minimize wrinkling and breaking of the electrode a heavier gauge (B. & S. 21) of roentgen-ray protection foil is used. In cutting the foil a substantial tongue is left at one end. A strong bull-dog clamp, made entirely of brass (gauge B. & S. 17), its jaws slightly turned in at the extreme edge, is applied to this tongue. The base of the clamp is equipped with hole and binding screw which provide for attachment of the lead wire. The essential features of this arrangement are displayed in the accompanying figure. A long, narrow strip of foil, designed to be wound

*From the Medical Clinic of Lakeside Hospital.
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†Cohn: Arch. Int. Med., 1920, xxvi, 105.

spirally about the arm, may be substituted for the foil plate shown in the figure. We have dispensed with the original rubber strip covering the metal. The electrode is applied over a cloth soaked in strong salt solution, and is held in place by means of a rubber bandage.

By this device the contact point is usually kept free from salt, but if renewing of surfaces is necessary it may be accomplished without removing the electrode from the limb. No sacrifice of the excellent qualities of the original electrode is involved in this modification.

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## THE ESTIMATION OF URIC ACID IN BLOOD*

By H. Brown, B.Sc., and G. W. Raiziss, Ph.D., Philadelphia, Pa.

THE ESTIMATION of uric acid, one of the important products of metabolism found in the blood, is not only regarded as a laborious procedure, but the accuracy of the various methods employed is still considered unsatisfactory by some investigators. Although much work has been done upon the subject since Garrod[1] developed his gravimetric method, little progress was made until Folin and Denis[2] introduced a colorimetric method dependent upon Salkowski's[3] principle of precipitating uric acid as silver urate. The amount of added uric acid recovered by their procedure averaged about 94 per cent. Benedict's[4] modification employing potassium cyanide, not only takes care of the excess of silver, thereby eliminating the hydrogen sulphide procedure, but also intensifies the color produced by the Folin and Denis reagent. This simplification gave a new impetus to research along similar lines. All the above methods, however, require large amounts of blood and concentration of the protein-free filtrate prior to precipitation of the uric acid.

In 1919, Folin and Wu[5] discovered a new blood-protein precipitant, tungstic acid, which yields a filtrate entirely free of proteins. In addition, but a small amount of blood is necessary and the filtrate may be analyzed for uric acid without further concentration. It is important to note in this connection, that they were able to recover quantitatively as much as 20 mg. of uric acid when added to 100 c.c. of blood, thus indicating that no adsorption by the precipitate occurs.

Another method of precipitating uric acid by means of a zinc salt in alkaline solution was suggested by Morris.[6] Recently, Morris and Macleod[7] in conjunction with this precipitant introduced a new reagent arseno-18-tungstic acid, as a substitute for phosphotungstic acid in the analysis of blood uric acid. Another variation which they introduced is the employment of sodium cyanide as the only alkali for development of color. Under these conditions the intensity of the final color produced is approximately three times that obtained by the original method of Folin and Wu.

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*Contribution from the Dermatological Research Institute. We are indebted to M. Falkov of this institute for valuable assistance in connection with this investigation.
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Still another modification of the latter procedure has recently been suggested by Pucher.[8] The essential variation consists in precipitating the proteins according to the original method but in addition heating for 10 minutes at 95° C. before filtering. In this way it is claimed, adsorption of uric acid by blood proteins is minimized, as evidenced by more complete recovery of added uric acid. Moreover, the time of filtration of the protein precipitate is greatly diminished.

Benedict[9] introduced a method for the direct determination of uric acid in the tungstic acid filtrate employing a combination of the reagents of Morris and Macleod, and Folin and Denis. The procedure in brief, is as follows: 5 c.c. of the protein-free filtrate are transferred to a test tube, while into another tube is introduced 5 c.c. of the uric acid standard containing 0.02 mg. of uric acid. To each are then added 5 c.c. of water, 4 c.c. of 5 per cent sodium cyanide solution, containing 2 c.c. of ammonia per liter and 1 c.c. of the arsenic phosphoric acid tungstic acid reagent. The contents of each tube are mixed by one inversion and immediately placed in water at 90° C. After 3 minutes, the tubes are immersed in cold water for 3 minutes and read in a colorimeter against the standard.

While engaged in clinical studies involving the daily analyses of many samples of blood for uric acid content, we were attracted to the latter method because of its speed, simplicity and requirement of small quantities of blood. The results varied so distinctly from those obtained by Folin and Wu's method, that an investigation of the limitations of Benedict's method was undertaken.

We proved to our own satisfaction, that, contrary to the suggestion of Pucher,[8] adsorption of uric acid does not take place during the precipitation of proteins by Folin and Wu's method if properly carried out. The following experiment substantiates this statement. Thirty c.c. of sheep's blood was divided into two portions of 10 and 20 c.c. To the 10 c.c. portion was added a solution of uric acid equivalent to 5 mg. of uric acid per 100 c.c. The proteins were then precipitated in each portion and the uric acid determined in the filtrates. Fifty c.c. of filtrate from the 20 c.c. sample of blood were then removed, sufficient uric acid solution added until its concentration in the total volume was equivalent to 5 mg. per 100 c.c. of blood, and the resulting solution analyzed for uric acid. The results are shown in Table I.

TABLE I

| URIC ACID IN ORIGINAL BLOOD | URIC ACID ADDED DIRECTLY TO 10 C.C. PORTION | URIC ACID ADDED TO FILTRATE OF 20 C.C. PORTION | URIC ACID RECOVERED FROM 10 C.C. PORTION | URIC ACID RECOVERED FROM FILTRATE OF 20 C.C. PORTION |
|---|---|---|---|---|
| mgm. / 100 c.c. | mgm. / 100 c.c. | mgm. / 100 c.c. | mgm. / 100 c.c. | mgm. / 100 c.c. |
| 0.0 | 5.0 | 5.0 | 5.0 | 5.3 |

The only modification adopted by us was the employment of Benedict's phosphate standard instead of Folin and Wu's sulphite standard. This produced more intense color, thereby permitting more accurate colorimetric

comparisons. In order to prevent adsorption, it is necessary to first add sufficient water so that after subsequent addition of the requisite amount of uric acid solution, the original volume of blood has been diluted by seven volumes of liquid. The precipitation of the proteins is carried out in graduated mixing cylinders. After the 10 per cent sodium tungstate has been added the whole should be thoroughly shaken. The ⅔ N-sulphuric acid must be added slowly and with constant shaking to prevent a temporary excess of acid and the formation of clumps of proteins. After all the acid has been added, the cylinder is very vigorously shaken to insure complete mixing. If these conditions have been observed, filtration can be started immediately. We invariably obtain perfectly clear filtrates which are neutral to congo-red paper.

Although, as pointed out by Benedict,[9] uric acid in the concentration existing in the tungstic acid filtrate cannot be precipitated by a silver salt in pure water, we have demonstrated in the following experiment that complete precipitation does occur in the above blood filtrate, due to the presence of other organic substances. After collecting the silver precipitate by centrifuging, the supernatant liquid was decanted and tested for uric acid with Benedict's reagent. It failed to reveal the presence of the slightest trace of uric acid despite the fact that this reagent is much more delicate than any previously used. We also proved that silver urate is completely decomposed by acid sodium chloride, for after treating the insoluble residue a second time with acid sodium chloride, and filtering, the filtrate gave no positive test for uric acid with the above reagent. In view of these facts, it is evident that the method of Folin and Wu permits of a fairly accurate determination of blood uric acid.

A comparison of the two methods was next made using the same protein-free filtrate. The results obtained are indicated in Table II.

TABLE II

| NO. | URIC ACID FOLIN AND WU | URIC ACID BENEDICT | NON-PROTEIN NITROGEN |
|---|---|---|---|
| | mgm. / 100 c.c. | mgm. / 100 c.c. | mgm. / 100 c.c. |
| 1 | 2.2 | 3.2 | 35 |
| 2 | 1.9 | 2.8 | 36 |
| 3 | 1.8 | 4.7 | 31 |
| 4 | 2.6 | 3.6 | 40 |
| 5 | 5.2 | 6.3 | 47 |
| 6 | 3.0 | 3.9 | 33 |
| 7 | 2.0 | 3.1 | 42 |
| 8 | 3.2 | 4.5 | 33 |
| 9 | 4.0 | 5.8 | 55 |
| 10 | 6.2 | 6.6 | 44 |

An examination of the figures shows that higher results were invariably obtained by Benedict's new method. Inasmuch as the Folin and Wu procedure involves many more manipulations than the Benedict method, there is a greater likelihood of analytical error which may possibly account for lower findings. But a series of experiments proved that equally satisfactory recoveries of added uric acid could be obtained by both methods as shown in Table III.

TABLE III

BY FOLIN AND WU'S METHOD

| NO. | URIC ACID IN ORIGINAL BLOOD | URIC ACID ADDED | TOTAL URIC ACID RECOVERED | ADDED URIC ACID RECOVERED |
|---|---|---|---|---|
| | mgm / 100 c.c. | mgm / 100 c.c. | mgm / 100 c.c. | mgm / 100 c.c. |
| 1 | 1.9 | 2.2 | 4.5 | 2.6 |
| 2 | 0.6 | 3.3 | 3.8 | 3.2 |
| 3 | 1.3 | 5.0 | 6.3 | 5.0 |
| 4 | 2.3 | 6.7 | 9.0 | 6.7 |
| 5 | 0.0 | 6.2 | 6.3 | 6.3 |
| 6 | 0.0 | 4.0 | 3.6 | 3.6 |
| 7 | 0.0 | 6.0 | 5.9 | 5.9 |
| 8 | 6.2 | 2.5 | 9.0 | 2.8 |
| 9 | 0.0 | 4.0 | 3.5 | 3.5 |
| 10 | 0.0 | 8.0 | 8.0 | 8.0 |

BY BENEDICT'S METHOD

| NO. | URIC ACID IN ORIGINAL BLOOD | URIC ACID ADDED | TOTAL URIC ACID RECOVERED | ADDED URIC ACID RECOVERED |
|---|---|---|---|---|
| | mgm / 100 c.c. | mgm / 100 c.c. | mgm / 100 c.c. | mgm / 100 c.c. |
| 1 | 3.2 | 4.0 | 7.1 | 3.9 |
| 2 | 3.7 | 1.33 | 5.0 | 1.3 |
| 3 | 4.1 | 4.0 | 7.8 | 3.7 |
| 4 | 4.1 | 10.0 | 13.5 | 9.5 |
| 5 | 4.4 | 4.0 | 8.7 | 4.3 |
| 6 | 1.2 | 6.0 | 7.0 | 5.8 |
| 7 | 1.2 | 8.0 | 8.8 | 7.6 |
| 8 | 1.2 | 10.0 | 11.0 | 9.8 |
| 9 | 0.0 | 4.0 | 4.1 | 4.1 |
| 10 | 0.0 | 8.0 | 8.0 | 8.0 |

Since we have already pointed out that no adsorption of uric acid by the blood proteins takes place, and also that the precipitation of silver urate in the Folin and Wu method is complete, we concluded that the higher results found by the Benedict method may be explained upon the assumption that the new reagent produces a color with other blood constituents. We tested the reagent with many organic substances, several of which were similar to those employed by Folin and Denis,[10] with the following results: 1. Aqueous solutions of amino acids such as glycine, alanine and leucine give slight color reactions. With a concentration of acid equivalent to 7 mg. of amino acid nitrogen per 100 c.c., there is obtained a color corresponding to that obtained by approximately 0.15 mg. of uric acid per 100 c.c. of blood. 2. Monohydric phenols containing no amino groups produce no color. Di- and tri-hydric phenols, however, with or without amino groups yield colors increasing in intensity with the number of hydroxyl groups. Thus, with one mg. of resorcin a color equivalent to about 0.04 mg. of uric acid is obtained, while that produced with one mg. of pyrogallic acid is equivalent to about 0.5 mg. uric acid. It is generally conceded that polyphenols are present in the blood.

The effect of these interfering substances, as Benedict has already pointed out, is greatly diminished in the presence of uric acid. Nevertheless there is no doubt that they influence the findings for this substance. Furthermore, the interference decreases with increase in uric acid content.

This can readily be seen upon attempting recoveries with animal blood. The following experiment will serve to illustrate this point. Rabbit's blood was divided into 4 equal parts; in one portion the uric acid was determined directly, the result obtained using Benedict's reagent was 3.7 mg. per 100 c.c. To the other 3 portions were added respectively, 4, 6, and 8 mg. of uric acid per 100 c.c. The total uric acid recoveries were 5.9, 7 and 8.3 mg. Actually, there is practically no uric acid in rabbit's blood, yet this is only indicated in the portion containing 8 mg. of uric acid. The action of the reagent, as Benedict points out, is probably selective; the uric acid reacting more readily than the interfering substances. The above experiment may serve as an explanation of the fact that the results obtained by Benedict's method check with those by Folin's method only in cases of high uric acid content.

Although Benedict has stated that the new method is only applicable to human blood, we found that animal bloods which, according to Folin and Wu's method contained no uric acid, when analyzed by Benedict's procedure, yielded positive findings. Table IV shows the equivalents in mg. of uric acid per 100 c.c. of blood corresponding to the respective intensities of color obtained.

TABLE IV

URIC ACID BY BENEDICT'S METHOD

| | mg. per 100 c.c. |
|---|---|
| Guinea pig 1 | 2.2 |
| Guinea pig 2 | 1.2 |
| Rabbit 1 | 2.1 |
| Rabbit 2 | 3.7 |
| Rabbit 3 | 1.9 |
| Rabbit 4 | 2.5 |
| Rabbit 5 | 2.5 |
| Rabbit 6 | 2.1 |
| Sheep 1 | 0.0 |
| Sheep 2 | 4.0 |

## CONCLUSIONS

1. Adsorption of uric acid does not take place in the precipitation of blood-proteins by the method of Folin and Wu.

2. Equally good recoveries of added uric acid can be obtained either by the method of Folin and Wu or Benedict.

3. Folin and Wu's method gives correct results for blood uric acid, while the high results found with Benedict's method are due to interfering substances.

4. Benedict's reagent is selective and yields results approximately similar to those of Folin and Wu only with high uric acid content.

5. Benedict's method, owing to its speed and requirement of small quantities of blood, can be used for routine clinical analyses, but is not to be recommended for research purposes.

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# A NEW METHOD FOR STERILIZING PROTEINS AND OTHER COLLOIDAL COMPOUNDS WITHOUT DENATURIZATION*

By C. A. Mills, M.D., Ph.D., Cincinnati, Ohio

THE preparation of protein solutions for medicinal use internally has always been attended with difficulty and more or less dissatisfaction on account of the inability to completely sterilize the product. This is obviated in part in making serum, antitoxin and thromboplastin preparations by using as nearly as possible aseptic technic in the handling of the materials, the use of only healthy animals, and the addition of a preservative to the final market product. It is realized by all that the cresol, phenol, chloretone or chloramine added as preservative will not destroy any spores that should happen to be present, but merely serve to inhibit growth, and possibly kill non-sporogenous organisms. In these protein solutions one is prevented from sterilizing with the more violent chemical reagents, such as formaldehyde, acids, alkalies, or oxidizing agents, because of the sensitivity of the product and the ease with which its physiological value may be destroyed. In making vaccines, tuberculin, etc., heat is used in varying degrees, so that complete sterility may be obtained, but most animal proteins, and, in all likelihood, most bacterial proteins are denaturized by heating above 56° C. in solution, so that we do not know how much more efficient vaccine therapy would be if this possibility of alteration in the proteins was removed.

It was with these points in mind, but more especially with the desire for a method of absolute sterilization of tissue fibrinogen (thromboplastin) solutions for subcutaneous injection, that we were led to devise the method given below.

*Principle.*—This depends on adding $HgCl_2$ to the protein solution in sufficient concentration to completely destroy all spores and organisms, followed, after complete sterilization, by the addition of sufficient sterile saturated NaCl solution to make about a 12 per cent concentration to decompose the mercury-protein precipitate, and finally the dialyzing out of the soluble double salt, using parchment paper bags and putting copper shavings outside to amalgamate with the mercury as it comes out, thus rendering the process irreversible.

*Method.*—Krönig and Paul[1] determined the time required for complete

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destruction of anthrax spores by varying concentrations of $HgCl_2$, finding complete sterility in 12-14 minutes with 1:65 parts $HgCl_2$ in watery suspension of the spores, 80 minutes with 1:500, and in 2 hours with 1:1000. For the same effect in protein solutions Behring and Nocht[2] found they must have a concentration of $HgCl_2$ of 1:200 for sterility in 80 minutes and 1:1000 for sterility in 24 hours. At various times NaCl and $NH_4Cl$ have been added with the $HgCl_2$ to prevent precipitation of the proteins, especially in preparations for use in sterilizing sputum and feces, but Krönig and Paul,[1] Scheuerlen and Spiro,[3] and others, showed that the addition of such salts markedly reduced the germicidal value of the $HgCl_2$, presumably through the formation of a double salt in which the mercury was bound in the complex ion.

Taking a solution of tissue fibrinogen containing about 1 per cent coagulable material, $HgCl_2$ was added to make a concentration of 1:500, the precipitated coagulant poured into a dialyzing tube and sealed to prevent the entrance of organisms. This was left for 2 hours at room temperature, after which time, sterile saturated NaCl solution was added to give a 12 per cent concentration and the tube left standing 15 minutes before immersing in the dialyzing beaker, in order to free all the mercury from the protein. Dialysis overnight at 3° C. with copper shavings in the water outside the tube removed the mercury so completely that the sulphide test failed to reveal a trace in the fibrinogen solution, the protein of which was now entirely back in solution and exhibiting its previous activity. The fibrinogen solution was now found completely sterile, whereas before, although it had stood in the ice box for three weeks after adding 0.3 per cent cresol, it constantly showed the presence of a spore bearing chain bacillus.

*Discussion.*—The author in a previous publication[4] showed that precipitation of the tissue fibrinogen with $HgCl_2$ in no wise lessened its activity in quickening blood clotting, after it had been again put into soluton by passing $H_2S$ through the suspended precipitate to form HgS. Kehoe[5] has recently found, also, that the activity of enzymes may be entirely suppressed by adding $HgCl_2$ to their solutions, but that their characteristic activity returns on adding proper concentrations of such salts as NaCl, KCl, etc., and that the mercury in these cases becomes dialyzable. Gustav Mann[6] demonstrated clearly many years ago the distinct dissolving action that NaCl has on Hg-protein precipitates through the formation of the light metal proteinate and the liberation of the mercury.

Although protein substances might be sterilized by $HgCl_2$, then, without permanent loss of their characteristic properties, the removal of the mercury from the solution was a difficult matter. It might be changed to the sulphide, but in the presence of the colloidal protein, this would not precipitate and could not be centrifuged out completely. After adding the NaCl to form the soluble and diffusible double salt of mercury, the mercury might be entirely removed from solution by merely immersing copper strips or gauze into the liquid. However, in most instances the salt added to redissolve the mercury would interfere with the use of the sterile product, so

that dialysis is required, either against running water or against a small volume of water with copper present to amalgamate with the mercury. In practice it would be most convenient to use such a volume of water as would leave 0.9 per cent NaCl solution inside the dialysis bag and thus have the sterile product all ready for injection. Such a process is readily carried out in an ice box and we may thus avoid the detrimental effects of exposure of sensitive materials to room temperature.

Other salts might be used with equal effectiveness for redissolving the mercury, all the halides of the light alkaline and alkaline-earth metals and ammonia acting in this way, potassium iodide perhaps working best, but it is simpler and just as efficient to use NaCl and leave the proper concentration in the preparation ready for injection.

It is to be noted that no single step in this procedure is at all new, but the combination of the different steps into a process for protein sterilization without permanent alteration, is new and offers some interesting and important possibilities in regard to the preparation of proteins for injection.

*Summary of Results.*—Protein solutions may be completely sterilized without alterations in their properties by letting stand the required length of time with the proper concentrating of $HgCl_2$, the precipitate being redissolved and the mercury rendered dialyzable by addition of NaCl. The mercury and salt may then be removed by dialysis, placing copper outside the dialyzing tube to amalgamate with the mercury.

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## METHOD OF DIVIDING THE URINARY BLADDER FOR EXPERIMENTAL PURPOSES*

By James A. H. Magoun, Jr., M.D., Rochester, Minnesota

DURING the investigation of various phases of the problem of infection of the urinary tract, the possibility of dividing the bladder into two portions, each in normal relation to its ureter, presented itself.

Female dogs kept under constant ether anesthesia were used. Aseptic technic was observed in all experiments. A low right rectus incision was made and the bladder isolated and packed off with gauze pads. Two stay sutures were placed in each side of the bladder and the organ was divided down its anterior wall into the urethra (Fig. 1). The ureteral openings were

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identified and a No. 5 ureteral catheter was introduced through each into the pelvis of the kidney. The posterior wall was then divided midway between the ureteral openings as far as the anterior had been divided. A soft rubber

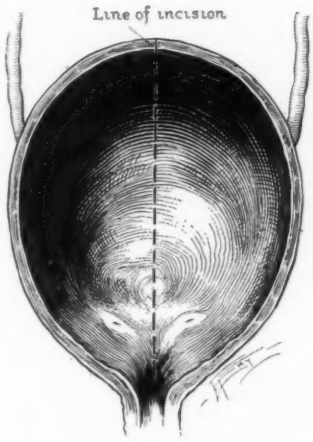


Fig. 1.—Line of incision for division of the bladder.

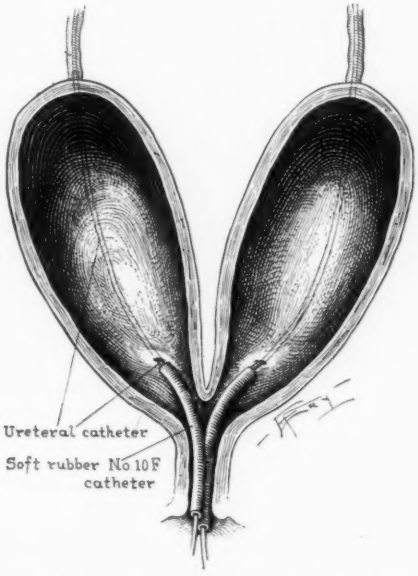


Fig. 2.—Operation completed. Bladder divided and catheters placed in ureteral openings and urethra.

Fig. 3.—Divided bladder, urethra, ureters and kidneys two months after operation.

No. 10 French catheter was placed into each side of the divided bladder and brought out of the external urethra. The respective ureteral catheters were then led through the urethral catheter to the external urinary opening (Fig.

2). The two segments of the bladder were closed over these catheters with two running sutures of No. 0 catgut. The surrounding fat was sewed over the lower portion of each segment (Fig. 3). The catheters were held in place by a few sutures of catgut just within the labia and were removed in from twenty-four to forty-eight hours. These catheters were advantageous in three ways: (1) as a means of identifying the ureter, (2) as a guide over which to sew the wall of the bladder, and (3) as a means of keeping the newly formed segments of the bladder empty.

The divided bladder permits the study of its cavity in normal relation to the ureter and kidney. Thus, one of the segments may be infected and the changes in the corresponding kidney and ureter may be noted. If obstruction to one segment below the entrance of its ureter is produced, the effect of obstruction at the outlet of the bladder may be studied. It is also possible by this means to study the comparative physiology of the two kidneys. The results of sudden decompression of the chronically distended bladder are now being observed by this method under the direction of the Sections on Urology and Experimental Surgery.

It is suggested that this method of placing catheters may be of some value in resections of the bladder in man.

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## A MODIFIED SAHLI HEMOGLOBINOMETER*

By William K. Trimble, M.D., Kansas City, Mo.

IN A LARGE hospital where routine hemoglobin estimations are required the method used is of considerable importance. The method must be reliable, quickly executed and free from extrinsic causes of error. Two forms of hemoglobinometers are largely in use, the Tallquist scale and the Dare. Both have a common objection in that the colors to be compared are in shades of red, probably the most difficult shades to compare. The Dare has a fixed and constant light while the Tallquist scale has the disadvantage of having to be read in very variable light conditions. Two workers may make quite widely different readings on the same sample of blood. Again, both are objectionable when a given sample has a brownish shade. The Sahli instrument is not practicable for rapid estimations but the colors to be compared are in the shades of brown. The modified Sahli here illustrated has the advantage of reliability, quick manipulation, simplicity, a fixed light and the reduction of the blood sample to a fixed color.

Select glass tubing of uniform caliber and of about 8 mm. outside diameter. The tubing is broken into 8 cm. lengths and sealed test tube fashion at one end. These are thoroughly cleaned and dried. Ten of these tubes are used in making the scale. A person is selected having a good 100 per cent

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hemoglobin. From the vein, without constriction, is drawn one c.c. of blood and placed into a container with a little dry sodium fluoride to prevent clotting. Into a small beaker place 5 c.c. of N/10 HCl and into this place 0.2 c.c. of the blood plus 0.02 c.c. for error. After the blood has completely darkened add 5 c.c. of glycerin. Into the first tube place one c.c. of the hematin hydrochloride glycerin mixture, into the second 0.9 c.c., into the third 0.8 c.c. and so on, decreasing the amount in each tube by 10 per cent, leaving in the tenth tube 0.1 c.c. Each tube is then made up to 1 c.c. by adding N/10 HCl and glycerin mixture. A scale of ten tubes is obtained, the first containing

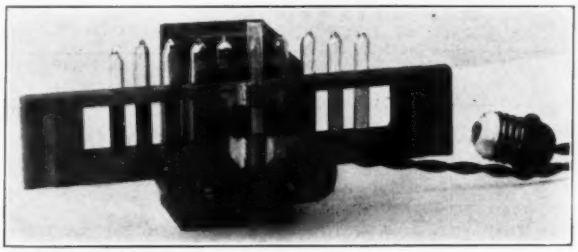

Fig. 1.

20 c.mm. of blood representing 100 per cent and the last 2 c.mm. or 10 per cent. The tubes are sealed in the blow flame. The scale is held in a sliding frame in a rack with a Spencer microlamp fastened to the back.

To make an estimation place 1 c.c. of N/10 HCl into one of the like tubes sealed at the end. Draw 20 c.mm. of blood into the standard Sahli pipette, place into the HCl with repeated rinsings "up and down." When completely darkened place in receptacle in front of the scale and read. As many tubes as needed may be carried in the blood counting tray. It is best to invert the scale daily. If accurately calibrated tubing can be obtained, a more perfect hematin hydrochloride solution may be made but the above is sufficiently accurate for routine work.

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## EDITORIALS

### *Present Status of the Use of Quinidine in Auricular Fibrillation*

SINCE the introduction of quinidine in cardiac therapy in 1918 by Frey, reports of about four hundred cases have appeared in the literature so that sufficient material has now accumulated to draw some conclusions as to the therapeutic value of the drug. Clark-Kennedy has reviewed the work on the first three hundred cases with the following conclusions.

In about one-half of the cases normal sino-auricular rhythm has been restored and in a small proportion of the unsuccessful cases, transient auricular flutter has been produced.

Quinidine acts by prolonging the refractory period and decreasing the conductivity of heart-muscle. During the administration of the drug the auricular rate falls and the ventricular rate rises, due to the lessening of fatigue in the auriculo-ventricular bundle. The transition from fibrillation to normal rhythm takes place usually through impure, and occasionally through pure flutter.

The number of successful cases runs approximately parallel with the dose of the drug employed. Thus Jenny, who gave as much as 46 gr. of the drug per diem, obtained 94 per cent of successes.

The action of quinidine in restoring normal rhythm is most successful in those cases in which auricular fibrillation is of short duration. On the other hand, many successful results have been observed in cases in which the disease was known to have been present for two or three years.

The degree of decompensation influences the action of quinidine unfavorably. But Benjamin and v. Kapff state that the degree of decompensation in which the patient is first seen is of less importance than the degree of compensation to which the patients can be brought by treatment. These authors therefore advise a preliminary course of digitalis in all cases exhibiting decompensation.

If the drug is discontinued, tendency to relapse seems to be the rule, and there is some evidence in the literature that if the drug is continued relapse is less likely to occur.

In the majority of successful cases there is some definite improvement in the objective and subjective clinical conditions of the patient. In many cases there is no change, and in a few cases the patients are definitely worse. In some cases symptoms and signs of failure, which did not disappear under digitalis, have yielded completely to quinidine therapy.

The commonest untoward symptoms of administration of the drug observed, are nausea, anorexia, vomiting, etc. The ventricular rate usually rises and symptoms and signs of increasing decompensation may occur.

Alarming symptoms—sudden pulselessness, cyanosis, apnoea, and unconsciousness—have been observed in a few cases. Frey has reported two such cases. But in neither case did death occur. It is interesting to note in this connection that Schott states that in animals intoxication with quinidine leads to sudden cessation of respiration. Several fatalities are recorded in the literature, but it is difficult to say in these cases whether the cause of death is definitely attributable to the drug. In addition to the two fatal cases reported by Hewlett and Sweeney, v. Bergmann reports two cases in both of which the heart was badly decompensated. One of these died four days after quinidine had been stopped, and the other shortly after a relapse into fibrillation had occurred and when quinidine was again being administered.

Benjamin and v. Kapff report two fatal cases, one of which died of broncho-pneumonia and the other a case fairly well compensated, but which reacted to quinidine with precordial pain and respiratory distress, and died soon after normal rhythm was restored. These authors also call attention to the risk of embolism, reporting one case of pulmonary infarction, and attribute the above case and the alarming symptoms in one of Frey's cases to this cause.

At present there seems to be no relationship between successful restoration of normal rhythm and the etiologic cause of the myocardial disease. All types of cases, rheumatic, degenerative, or associated with exophthalmic goiter, seem to react or fail to react in approximately equal proportions.

Mechanical efficiency of the heart depends upon three chief factors: first, the functional efficiency of the myocardium as a source of kinetic energy; second, the efficiency of the valves in preventing regurgitation of blood and the patency of the valves in offering a minimum of resistance to flow through them; and third, the maintenance of an orderly rhythmic contraction of its component chambers. Interference with any of the three factors may produce a decided handicap in the work of this organ. In cases of fibrillation there usually already exists either myocardial disturbance or valvular disturbance (mitral stenosis) or both. In such cases the reserve cardiac force is already considerably diminished and the advent of fibrillation usually brings cardiac failure. This is of course directly due to the resultant added strain on the heart muscle. Ventricular filling is rendered less efficient by the failure of auricular contraction; there is considerable wastage during the ventricular systole due to the irregular stimulation, and the ventricular rate is no longer controlled by the sino-auricular node.

In certain cases the onset of fibrillation does not bring on heart failure. These are the individuals in whom the myocardium is relatively little affected and the reserve force is still sufficient to care for the added burden, or those who because of previous rest in bed have been carrying a minimal load, or again, those in whom a partial heart block has occurred.

The two methods of treating fibrillation now in use are with digitalis and quinidine. With digitalis therapy a partial heart block is produced so that the weaker auricular stimuli are shut off from the ventricle. A digitalized heart is not functionally efficient because the auricles are still fibrillating and the ventricle remains irregular. The blood is incompletely discharged from the auricles and many of the ventricular contractions are incomplete.

With quinidine a normal rhythm is established, the heart is again dominated by its pace-maker, and the heart rate can be adjusted through nervous reflexes to the rate of diastolic filling and the peripheral blood pressure. Theoretically at any rate, this method of treatment should be more economic of cardiac reserve power.

However, we must bear in mind that this will not relieve the difficulty entirely. Only one of the three factors dominating cardiac functional capacity has been remedied and myocardial or valvular disease usually continues to complicate the picture.

The action of quinidine in fibrillation is best interpreted in terms of Lewis' recent theory of circus movement discussed in a recent editorial in this journal. Quinidine lengthens the refractory period of the auricle from fifty to one hundred per cent. According to the theory of circus movement, lengthened transmission intervals would slow the auricular rate and the longer refractory period would have a similar action, for if sufficiently prolonged, it would divert the wave upon a longer path. Theoretically, normal cardiac rhythm can be restored in flutter or fibrillation by closure of the gap between the crest and wake of the circulating wave. This gap may be closed (1) by an increase in the duration of the refractory period, (2) by an increased rate of conduction, or (3) by a shortening of the path of the circus movement.

As we have seen, quinidine increases the refractory period but it slows the rate of conduction. If these two changes equalize each other, the drug will have no effect in closing the gap. It is only when the former exceeds in amount the latter that closure may be anticipated, and when this does occur, the heart rhythm again becomes regular.

Quinidine acts not alone on the conduction apparatus in the auricle, but also on the vagus nerve with a partial paralytic action, and on the ventricular musculature. This latter action follows diminution of the low grade heart-block which usually accompanies fibrillation and results in an increase in the ventricular rate. Rarely the ventricular rate falls, due to an increase in the P-R interval. Thus we can understand the occurrence of varying responses in different individuals according to which action of the drug predominates.

White points out that chronic heart disease, especially with chronic mitral stenosis and auricular fibrillation of more than one year's duration, is in general a contradiction for quinidine therapy, first because of the danger of embolism when the auricles again start their normal contraction; and second, because in such hearts the rhythm returns to normal only in a small proportion of cases, and even in these there is a decided tendency to relapse. It is in auricular fibrillation of recent origin without heart failure or pronounced mitral stenosis that quinidine therapy seems at present to be especially indicated.

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—*W. T. V.*

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## *Blood Stains*

THE most commonly used blood stains (of which Wright's stain is the best known example) are combinations of eosin and methylene blue. The precipitate formed when these two dyes are properly combined is dissolved in methyl alcohol in its preparation for use. Before the war, Grübler stains were used for this purpose and the solvent employed was Merck's "reagent alcohol."

Since the supply of foreign dyes was cut off in this country blood stains put upon the market have been very largely unsatisfactory. Some investigators seem to feel the methylene blue at fault and others the eosin; but in light of investigations that have recently been carried on showing the high quality of most American eosins and methylene blues, it would not seem as though the fault could lie primarily with them. There seems more reason to suspect the solvent, inasmuch as Merck's "reagent alcohol" is no longer available. This was such a logical conclusion that two concerns, the Will Corporation and the National Anilin Company, working independently on blood stains, gave special attention to the solvent used, and have prepared

specially purified methyl alcohols for use in blood stains. Each of these companies at present puts on the market both the blood stains prepared with these solvents and also a methyl alcohol which is indicated on the label to be intended for blood stains. The products of both companies seem to be entirely satisfactory, favorable reports having been received from Dr. J. H. Wright himself.

Although both of these products are satisfactory, special reference must be made to the National Anilin products, because the Will Corporation is not primarily a manufacturer of stains and has in the past been manufacturing only those that they have been unable to obtain in good quality elsewhere. As their policy is to discontinue such manufacture whenever satisfactory sources are located, it is quite possible that they may now discontinue their own blood stains in favor of those of the National Anilin Company. Whatever happens in this particular, however, it is plain that satisfactory blood stains are now available and both of these companies deserve much credit for what they have done in the matter.—*H. J. Conn, Chairman, Commission on Standardization of Biological Stains.*